VOLUME XVI MAY, 1933 NUMBER 3

# JOURNAL OF DAIRY SCIENCE

PUBLISHED BY THE
AMERICAN DAIRY SCIENCE ASSOCIATION

## *Contents*

*Some Factors Influencing the Crystallization of Lactose in Ice Cream.* RANDALL WHITAKER ..... 177

*A Study of the Phosphorus Requirement of Dairy Cattle.* C. F. HUFFMAN, C. S. ROBINSON, C. W. DUNCAN, L. W. LAMB, and M. F. MASON ..... 203

*Laboratory Studies on the Chemistry of Soft Curd Milk.* S. M. WEISBERG, A. H. JOHNSON, and E. V. MCCOLLUM ..... 225

*An Improved Method for Preparing the Serum of Butter for pH Determination.* K. G. WECKEL ..... 249

*Calcium and Phosphorus of Cheese Made Under Controlled Conditions.* R. B. MCCAMMON, W. J. CAULFIELD, and M. M. KRAMER ..... 253

*The Use of Vegetable Stabilizers in Ice Cream.* W. J. CAULFIELD and W. H. MARTIN ..... 265

*Clarence Henry Eckles* ..... 273

*Committee Reports: Methods of Analyzing Dairy Products—The Bacteriological Analysis of Ice Cream; The Microbiological Analysis of Butter; The Chemical Analysis of Butter* ..... 277

*Association Announcements and News* ..... 307

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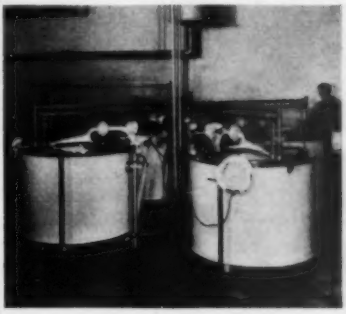

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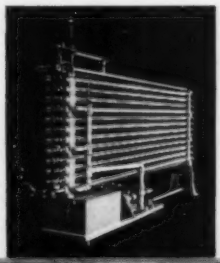

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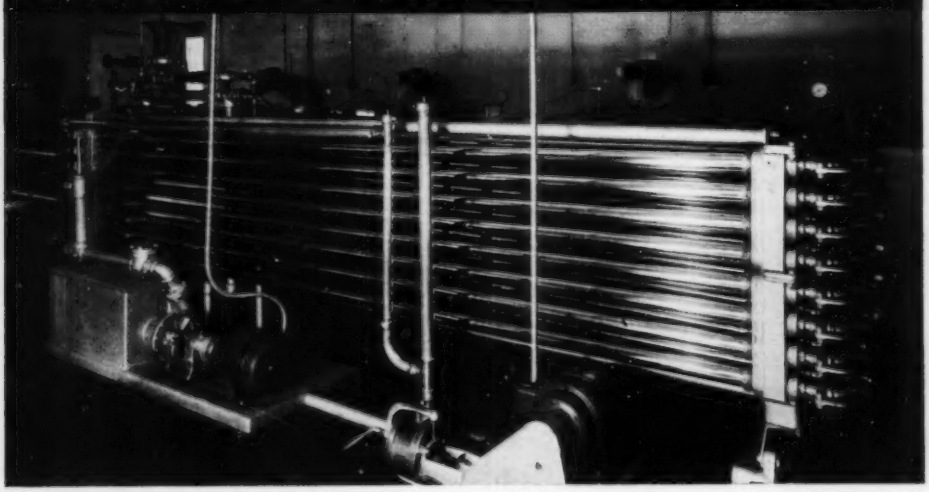

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JOURNAL OF DAIRY SCIENCE

VOLUME XVI MAY, 1933 NUMBER 3

# SOME FACTORS INFLUENCING THE CRYSTALLIZATION OF LACTOSE IN ICE CREAM

RANDALL WHITAKER

*Research Laboratories of National Dairy Products Corporation, Inc., Baltimore, Maryland*

The crystallization of lactose in ice cream is of interest as the defect known as sand is caused by the presence of lactose crystals of sufficient size that they can be detected by the tongue on the roof of the mouth. This was first shown in 1920 and 1921 by Bothell (1) and Zoller and Williams (2). Following these early papers many publications have appeared on the subject of sandy ice cream, the conditions which promote this defect and suggested methods of avoiding sand. The physical chemistry of lactose crystallization has also been studied fairly thoroughly. Prior to discussing any experimental work, it seems advisable to review the known facts and theories regarding the crystallization of lactose in ice cream.

Ice cream mix is usually aged at 1.6° to 4.4° C. (about 35–40° F.) and it is at this temperature that most ice cream enters the freezer. Rahn and Sharp (9) state that a saturated lactose solution will contain about 11 per cent lactose at this temperature, hence in all ice cream mixes of average composition the lactose will be in solution, as the concentration of lactose in unfrozen mix very rarely exceeds 11 per cent. It sometimes happens that lactose is crystallized in one or more of the ingredients used in the manufacture of ice cream but the pasteurization process assures complete solution of all the lactose in ice cream mix.

## *Condition of Lactose in Ice Cream Mix During the Freezing and Hardening Process*

During the freezing process while the ice cream mix is being slushed and air is being incorporated, part of the water is frozen into ice. Since nothing but water goes to form ice it follows that the unfrozen solution containing the water soluble constituents, such as sucrose, lactose, the salts, gelatin and the like, is concentrated in proportion to the amount of ice which is formed. Table 1 gives data concerning the amount of ice which separates at various temperatures in three mixes of varying composition. This was

Received for publication July 5, 1932.

TABLE 1

*Lactose concentration and freezing points of mixes at various stages in the freezing process*

*Values calculated from the work of Leighton (10)*

| PARTS OF ICE FROZEN | PARTS UNFROZEN WATER IN THE MIX | | | PARTS LACTOSE PER 100 PARTS UNFROZEN WATER | | | TEMP. OF THE MIX—°C. (FREEZING POINT) | | | TEMP. OF THE MIX—°F. (FREEZING POINT) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C | A | B | C |
| 0 | 63.75 | 61.75 | 59.75 | 8.6 | 10.6 | 12.8 | −2.2 | −2.4 | −2.7 | 28.1 | 27.6 | 27.1 |
| 5 | 58.75 | 56.75 | 54.75 | 9.3 | 11.5 | 13.9 | −2.4 | −2.6 | −3.0 | 27.7 | 27.2 | 26.6 |
| 10 | 53.75 | 51.75 | 49.75 | 10.1 | 12.6 | 15.3 | −2.5 | −2.9 | −3.2 | 27.4 | 26.7 | 26.3 |
| 15 | 48.75 | 46.75 | 44.75 | 11.2 | 14.0 | 17.0 | −2.9 | −3.3 | −3.7 | 26.8 | 26.1 | 25.3 |
| 20 | 43.75 | 41.75 | 39.75 | 12.4 | 15.7 | 19.2 | −3.2 | −3.7 | −4.1 | 26.2 | 25.4 | 24.6 |
| 25 | 38.75 | 36.75 | 34.75 | 14.3 | 17.8 | 22.0 | −3.7 | −4.3 | −4.9 | 25.4 | 24.3 | 23.1 |
| 30 | 33.75 | 31.75 | 29.75 | 16.2 | 20.6 | 25.6 | −4.3 | −5.5 | −5.9 | 24.2 | 22.9 | 21.4 |
| 35 | 28.75 | 26.75 | 24.75 | 19.0 | 24.4 | 30.8 | −5.3 | −6.2 | −7.3 | 22.6 | 20.9 | 18.8 |
| 40 | 23.75 | 21.75 | 19.75 | 23.0 | 30.0 | 38.6 | −6.3 | −7.9 | −9.7 | 20.3 | 17.8 | 14.5 |

A—Mix contained 12% fat, 10% milk-solids-not-fat, 14% sugar, 0.25% gelatin.
B—Mix contained 12% fat, 12% milk-solids-not-fat, 14% sugar, 0.25% gelatin.
C—Mix contained 12% fat, 14% milk-solids-not-fat, 14% sugar, 0.25% gelatin.

calculated from the work of Leighton (10). Rahn and Sharp (9) interpolated and extrapolated the data by Hudson (5) regarding the final solubility of lactose at various temperatures. When a concentration of 11.9 parts of lactose in 100 parts of water is obtained at 0° C. (32° F.), the solution is saturated to lactose. It will be seen from table 1 that the lactose in the unfrozen water in ice cream mix in the freezer reaches and actually exceeds the saturation point. The concentration increases with decrease in temperature, so that in ice cream at cabinet and hardening room temperatures the lactose is in a highly supersaturated condition. It is evident that there are forces working to prevent the lactose from crystallizing in ice cream and that these forces are sufficiently potent to delay crystal formation despite the high supersaturated condition of lactose in commercial ice cream.

In order to understand why the lactose does not crystallize as soon as ice cream is frozen, a discussion of some of the physical and chemical properties of supersaturated lactose solutions seems in order at this time. The discussion will be extended as necessary to include conditions as found in ice cream.

*Properties of Supersaturated Lactose Solutions*

Leighton and Peters (11) first pointed out the significance of the properties of supersaturated lactose solutions in relation to the sand defect in ice cream. When a saturated lactose solution is cooled or concentrated in any way so that the lactose becomes supersaturated, crystallization does or does not take place, depending on the degree of supersaturation. Three concentration areas or states are known, viz: metastable, labile and glassis.

*Metastable area:* This area occurs directly below the saturation point. If a supersaturated lactose solution is held in this area it will not crystallize under normal conditions. Factors which generally operate to cause crystallization, such as agitation, jarring, seeding, and the like, do not lead to copious crystallization unless the action is very intensified.

Leighton and Peter (11) worked out the super-solubility curve for pure lactose at various temperatures above the freezing point. This curve corresponds to the lower edge or boundary of the metastable area described above. It would be difficult to determine accurately the position of this boundary in ice cream because of the ice which would form and because of the influence various substances like protein, sugar, salts and fat might have on the position of the line.

*Labile area:* As the concentration of lactose in a supersaturated solution is increased beyond the metastable area the lactose is said to enter the labile area. As the word indicates supersaturated lactose solutions in the labile area are unstable and are subject to spontaneous nuclei formation and rapid subsequent crystal growth. Crystals formed from supersaturated solutions

in this state are frequently irregular in shape. This is probably caused by the rapid deposition of lactose on the nuclei. Clustering of crystals is another characteristic noted under these conditions.

As mentioned previously, Leighton and Peter (11) established the metastable/labile boundary in pure lactose solution above the freezing point but no one has attempted to define the concentration range of the labile area for lactose. In the manufacture of spray dried whey it has been found that if the moisture content is 7 per cent or more crystallization of lactose takes place. As dried whey contains about 70 per cent lactose it follows that in this product the labile area of the lactose extends to about 11 per cent moisture in the lactose. Leighton (10) has pointed out the desirability of expressing the lactose concentration as parts of lactose per 100 parts water. On this basis in dried whey the concentration of lactose in the labile area ranges from approximately 45 to 890 parts lactose to 100 parts water at room temperature. It must not be concluded that this value applies to lactose in pure solution as the salts and albumin in whey probably have an influence on the position of this area. However, these calculations may be considered as approximate and indicate that this area covers a wide range.

*Glassis area:* Further concentration of a non-crystallized supersaturated lactose solution forces the system out of the labile area into what is called the glassis area. In this state crystallization fails to take place. The lactose solution or syrup becomes so concentrated that it forms a glass or what may be called a solid solution. Molecular movement is at a minimum. There is practically no diffusion throughout the mass. It may even be that the molecules are not capable of arranging or orienting themselves to form crystals or to continue crystal growth. Whether the lactose in ice cream is ever found in the glassis state is problematical, as there is no accurate set of data to show the amount of metacryotic liquid present at low temperatures.

### *Isomeric Forms of Lactose*

The crystallization of lactose in ice cream is complicated by the fact that lactose exists in more than one form. Organic compounds which contain asymmetric carbon atoms are subject to stereoisomerism. In the case of lactose two isomeric forms are known, namely, alpha and beta lactose. Troy and Sharp (6) give a good discussion of the rôle played by alpha and beta lactose in the crystallization of this sugar in ice cream.

In a non-saturated solution of lactose in water the alpha and beta forms are in equilibrium. The ratio of the two forms varies slightly with temperature. According to Rahn and Sharp (9) at 0° C. (32° F.) the equilibriated solution contains 62.25 per cent beta lactose and 37.75 per cent alpha lactose, which gives an equilibrium constant of 1.65. It makes no difference which of the two forms is dissolved as in solution alpha changes

to beta and beta changes to alpha with relative ease until the equilibrium ratio is established. The rate of conversion is almost instantaneous at 75° C. (167° F.) and above, but at lower temperatures a time factor appears. At sub-freezing temperatures the rate becomes very slow.

Sandy ice cream is caused by the presence of alpha lactose crystals. If a solution containing alpha and beta in equilibrium is concentrated so that the solution becomes supersaturated to the alpha form, alpha rather than beta lactose crystallizes as this form is less soluble. In order to have more alpha crystallize some of the lactose which is in the beta form must change to alpha to maintain the equilibrium ratio. The rate of conversion of beta to alpha is very slow at the temperature of hardened ice cream and since this reaction may take place less rapidly than the rate of alpha crystallization, it becomes the factor which governs the rate at which lactose is deposited in the solid state. The rate of conversion of beta to alpha decreases with temperature, hence the colder the solution the less rapid will be the formation of crystallized alpha lactose.

In ice cream mix alpha and beta lactose are in equilibrium as the pasteurization temperature insures the rapid conversion of the two forms. The equilibrium is maintained after cooling and during the aging process, as in average ice cream mix the lactose in solution in the water of the mix does not become saturated to alpha. In the freezer, however, some water is removed as ice, the solution becomes supersaturated to alpha lactose, and there is a tendency for alpha to crystallize. Actually, no alpha crystallizes as there is an insufficient amount of alpha present to immediately form crystals. The conversion of beta lactose to alpha lactose in the solution occurs only when the equilibrium is disturbed by the crystallization of alpha lactose. The lactose solution in the mix is probably in the metastable state, which, as has been previously noted, is not conductive to crystallization.

### *Rate of Diffusion in Ice Cream at Low Temperatures*

Peter (13) has shown that "the solubility of lactose in nearly saturated sucrose solutions is reduced to approximately one half its value in water." The freezing of water into ice in ice cream causes a further concentration of the lactose insuring a highly supersaturated solution of lactose in ice cream at low temperatures. Peter (13) in a discussion of the properties of nearly saturated sucrose solutions points out that at low temperatures such solutions become extremely viscous, which factor "creates an influence which opposes and materially retards the separation of lactose." There is evidence to show that in ice cream of average composition the sucrose solution actually does become saturated, probably at about $-13.3°$ to $-11.1°$ C. (8 to 12° F.). The very high viscosity of saturated and supersaturated sucrose solutions at these low temperatures reduces the rate of diffusion of lactose to a marked degree. Since diffusion is necessary for crystal for-

mation and growth, it appears that this factor is of great importance in the rate of lactose crystallization in ice cream.

## METHODS OF DETECTING CRYSTALLIZED LACTOSE IN ICE CREAM

It has been demonstrated by various workers that ice cream is said to be sandy when it contains lactose crystals sufficiently large to be felt between the tongue and the roof of the mouth. Individual crystals or clusters of crystals having a total length of 30 microns or more produce a distinctly noticeable sandy condition, while crystals of 12 microns or less are not felt in the mouth (8). It is possible under certain conditions to have an appreciable quantity of lactose crystallized in ice cream and yet have a product which is not sandy to the tongue, providing the crystals present are very small.

Four methods of detecting lactose crystallization in ice cream were studied. Two methods were employed in this investigation. Of the two remaining methods one was discarded after preliminary trials showed it to be unreliable and the other discarded because of the complicated technique required. For the sake of record, however, all four methods will be briefly discussed.

### *Seeding Test*

Hudson (5) discovered that if a small amount of a substance, either liquid or solid, containing crystallized alpha lactose, was added to a supersaturated solution of pure lactose in water, extensive and visible crystallization took place in a short period of time. If, however, the added substance did not contain alpha lactose crystals no crystallization occurred. In the former case the lactose crystals acted as seeding nuclei, causing copious crystallization while in the latter case no crystallization resulted due to the absence of seeding nuclei.

This test, known as Hudson's Seeding Test, was used on several samples of ice cream with the result that almost every sample showed positive, although many were known to be free from lactose crystals. It was believed that there were present in the ice cream and mix other substances which induced crystals to form in the supersaturated lactose solution. Troy and Sharp (6) also found Hudson's Seeding Test to be unreliable when used on ice cream.

### *Polariscopic Method of Detecting Crystallized Lactose*

Lactose, in common with many organic compounds, has the property of rotating the plane of polarized light passing through its solution. In the case of lactose where two forms are possible, namely the alpha and beta forms, there will be a corresponding number of specific rotations. The specific rotation is a measure of the degree to which plane polarized light

is rotated by a substance. The specific rotation of the various forms of lactose are given by Whittier (7) as follows: alpha + 89°, beta + 35°, and alpha – beta (equilibrium mixture) + 55.5°. Thus a freshly prepared solution of alpha in water will have a specific rotation of + 89°. As the solution is allowed to stand the reading will be observed to decrease as a portion of the alpha changes to beta. The rotation will finally remain constant at + 55.5°, which is the value of the equilibrium mixture of alpha and beta. In like manner, if the beta form is dissolved in water the first value will be + 35°. This will change slowly to + 55.5° as equilibrium is reached.

In another section of this report a detailed discussion of the alpha and beta forms of lactose in ice cream is given. These two forms of lactose are in equilibrium in all unfrozen mix and in freshly made ice cream, hence the lactose in a clarified solution of ice cream mix will have a specific rotation of + 55.5°. If, however, conditions are such that alpha lactose has crystallized, as in sandy ice cream, a reading between + 55.5° and + 89° will be observed, which value will slowly change to + 55.5° on standing. Thus, when a given sample of ice cream is examined polariscopically and a reading above that of + 55.5° is obtained, which decreases on standing to this constant, it is proof that some crystallized alpha lactose is present in the ice cream. Troy and Sharp (6) used this method in following lactose crystallization in ice cream.

This method of determining lactose was found to have certain disadvantages. It was (1) time consuming, as the mix had to be clarified with some protein precipitant prior reading in the polariscope, (2) necessary to have accurate temperature control while operating the polariscope, (3) necessary to conduct the test in a well equipped laboratory because of the rather precise technique, (4) expensive since a polariscope costing an appreciable amount of money was required.

The advantages of the test were (1) dependable; and (2) fairly sensitive, detecting small amounts of crystallized lactose.

### *Microscopic Method of Detecting Crystallized Lactose*

Heretofore the use of the microscope in detecting crystallized lactose in ice cream has been somewhat limited due to the difficulty attending the preparation of the frozen specimen. To prevent solution of very small crystals the work had to be done at a cold temperature. Following suggestions made by Herrington (20), a simplified technique was worked out whereby this method could be used at room temperatures. As this method is not recorded in the literature, it seems desirable to discuss it in some detail at this time.

A lactose solution is prepared which is saturated to lactose at 35–37° C.

(95–99° F.). This solution is carefully filtered at that temperature to remove any crystals which may be present and allowed to cool to room temperature.

A drop of this supersaturated solution is placed on a glass slide on the stage of a polarizing microscope. A small portion of the frozen ice cream to be tested is introduced into the lactose solution on the slide, where it promptly melts. The sample plus lactose solution is stirred to form a uniform mixture. A cover glass is then applied and the preparation viewed through crossed nicols.

The lactose crystals in the ice cream, provided they are present, do not dissolve when the solution is warmed to room temperature because they are suspended in a saturated lactose solution. The dilution effect is counterbalanced by having the lactose solution saturated at a temperature slightly higher than that at which the work is done. The slide is best examined immediately although crystallization does not take place at once as the rate of alpha crystallization is comparatively slow.

It is possible by using 200 magnification to detect very small crystals. The crystals appear as small points of light which change in intensity as the stage of the microscope is revolved. No other substance present in ice cream will act in this manner, hence it can be said that all preparations which exhibit this phenomenon have lactose crystals present. It is not always possible to detect the crystals when the slide is viewed through parallel nicols or with the ordinary microscope. The rotation of the stage assists greatly in detecting the presence of the crystals, although it is not absolutely necessary.

The microscopic test was found to be very practical. It was used almost exclusively throughout this research as a means of detecting the first sign of lactose crystallization. It was proved in many trials that when crystals were detected with the microscope the ice cream never failed to become sandy to the tongue at some later date.

The advantages of this method over the polariscope, the other most dependable test, are as follows: (1) the microscopic method was found to be more sensitive, detecting lactose crystallization one or more days earlier than the polariscopic method; (2) the microscopic test was more rapid, results were obtained in 2–3 minutes, whereas the polariscopic method required at least one hour; (3) the results obtained with the microscope were never debatable, whereas there was often a question about the change in specific rotation of the solution in the polariscopic method; (4) the test was more practical, as it was readily conducted in any ice cream plant; (5) the technique employed was very simple in comparison to the polariscopic test.

### *Detection of Lactose Crystals in the Mouth*

Large lactose crystals are easily detected in the mouth, especially when the ice cream is rubbed between the tongue and the roof of the mouth or ground between the front teeth. The low solubility of lactose is responsible for the presence of the crystals after the ice cream has melted in the mouth. At certain times the crystallized lactose creates a tickling sensation at the back of the mouth and there is often a desire to cough slightly or to clear the throat. Inexperienced tasters may at first confuse coarseness in ice cream as caused by excessive ice crystallization with the grittiness of lactose crystals, but the persistent character of the latter makes it so much more noticeable that sandy ice cream is readily distinguished from coarse or icy ice cream. As mentioned elsewhere in this report, lactose crystals must be 30 microns or larger to be easily detected in the mouth. When crystals which vary in size from 15 to 20 microns are present in large numbers the sand defect can be frequently detected by the mouth, according to Fujumoto (8).

Comparison of the three useful methods of detecting crystallized lactose in two samples of ice cream is shown in table 2. It will be observed that the microscopic method detected smaller crystals than were felt in the mouth and was the most sensitive method used. It was possible to predict by the use of the microscopic test the sand defect several days before it was noticed organoleptically.

## FACTORS INFLUENCING LACTOSE CRYSTALLIZATION IN ICE CREAM

The literature contains discussion of many of the factors which have been found to influence lactose crystallization in ice cream. Other factors, not mentioned in any published work as far as could be ascertained, have been studied during the course of this investigation. There follows a discussion of some of the factors which influence lactose crystal formation in ice cream as recorded in the literature and as found during the perusal of this problem. Record is also made of factors which gave promise of affecting lactose crystallization but which were later found through experimentation to have no significant bearing on the problem.

### *Composition of the Mix*

Bothell (1) in 1920 was probably the first to call attention to the composition of the mix as a factor causing sandy ice cream. Nearly every published discussion of the sand defect includes mix composition as one of the chief factors. It appears obvious that the greater the quantity of lactose present in the mix, the greater will be the tendency of lactose to crystallize and for the ice cream to become sandy.

In most publications the quantity of lactose is not mentioned directly but rather the percentage of the solids-not-fat in the mix. This is rather

(95–99° F.). This solution is carefully filtered at that temperature to remove any crystals which may be present and allowed to cool to room temperature.

A drop of this supersaturated solution is placed on a glass slide on the stage of a polarizing microscope. A small portion of the frozen ice cream to be tested is introduced into the lactose solution on the slide, where it promptly melts. The sample plus lactose solution is stirred to form a uniform mixture. A cover glass is then applied and the preparation viewed through crossed nicols.

The lactose crystals in the ice cream, provided they are present, do not dissolve when the solution is warmed to room temperature because they are suspended in a saturated lactose solution. The dilution effect is counterbalanced by having the lactose solution saturated at a temperature slightly higher than that at which the work is done. The slide is best examined immediately although crystallization does not take place at once as the rate of alpha crystallization is comparatively slow.

It is possible by using 200 magnification to detect very small crystals. The crystals appear as small points of light which change in intensity as the stage of the microscope is revolved. No other substance present in ice cream will act in this manner, hence it can be said that all preparations which exhibit this phenomenon have lactose crystals present. It is not always possible to detect the crystals when the slide is viewed through parallel nicols or with the ordinary microscope. The rotation of the stage assists greatly in detecting the presence of the crystals, although it is not absolutely necessary.

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In most publications the quantity of lactose is not mentioned directly but rather the percentage of the solids-not-fat in the mix. This is rather

TABLE 2

*Comparison of three methods of detecting lactose crystallization in two samples of ice cream*

| DAYS IN STORAGE | MISCROSCOPIC | | POLARISCOPIC | | ORGANOLEPTIC | |
|---|---|---|---|---|---|---|
| | A | B | A | B | A | B |
| 11 | − | − | − | − | − | − |
| 12 | − | − | − | − | − | − |
| 13 | − | − | − | − | − | − |
| 14 | + | − | − | − | − | − |
| 15 | + | + | + | − | − | − |
| 16 | + | + | + | + | + | − |
| 17 | + | + | + | + | + | + |

The samples (A and B) were removed from the cabinet for 15 minutes daily to hasten lactose crystallization.

unfortunate as it fails to present a true picture of the conditions which exist in any particular mix. A better index of the degree of lactose concentration may be obtained by considering the ratio of lactose to water. The treatment which the ice cream receives should also be given attention in determining the amount of milk-solids-not-fat to use in a mix to avoid the sand defect. The proper lactose to water ratios which should obtain in mixes as calculated from Sommer's (17) factors are presented in table 3.

TABLE 3

*Maximum percentage of milk-solids-not-fat to avoid sand defect under various conditions*

| TREATMENT GIVEN THE ICE CREAM | % SOLIDS-NOT-FAT TO USE WITH 12% FAT + 15% SUGAR | LACTOSE TO WATER RATIO | SOMMER FACTOR F |
|---|---|---|---|
| Ideal conditions: Rapid turnover. Ice cream sold in few days | 11.3 | 1: 11.0 | 6.4 |
| Fair conditions: Stocks held in cabinets two weeks | 10.5 | 1: 11.9 | 6.9 |
| Poor conditions: Stocks held in cabinets four weeks | 10.0 | 1: 12.6 | 7.3 |

He has developed a simple formula for establishing the amount of solids-not-fat to use in any given mix which will not cause sandiness under the conditions prescribed.

$$\text{Maximum per cent of solids-not-fat to use} = \frac{(100 - X)}{F}$$

where $X$ = sum of all other solids except milk-solids-not-fat
$F$ = factor as given in table 3.

Several investigators (3) (4) (8) (15) have shown so conclusively that there is a direct relationship between the percentage of solids-not-fat in the mix to the rate at which the sand defect appears that there was no need of further experimentation on this phase of the problem. It is surprising, however, to note the lack of a satisfactory explanation for this observation as recorded in the literature. At first thought it would appear that the so-called "danger line" or limit of 10 to 12 per cent solids-not-fat described by many workers was merely due to the formation of a supersaturated solution when this quantity of serum solids was used in ice cream. A closer and more intelligent study of the facts, however, indicates that this explanation is inadequate. A discussion of a possible explanation for this observation does not seem amiss at this time.

It has been shown elsewhere in this report that lactose exists in ice cream in a supersaturated state. It was further shown that lactose exists in one of three states or areas of supersaturation. As indicated, no definite concentration or temperature range can be ascribed to the three states of lactose in supersaturated solution as it exists in ice cream. It is likely that the colloids, sucrose, and salts of ice cream have an influence on the solubility of lactose and on the metastable/labile/glassis boundaries. The action of these substances adds to the complexity of the problem and to the difficulty of formulating a complete explanation for the peculiar behavior of lactose as it crystallizes in ice cream under various conditions.

As previously pointed out, agitation of a supersaturated lactose solution in the metastable state does not lead to immediate crystallization, whereas crystallization is promoted if the lactose is in the labile state. Lactose should not, therefore, crystallize readily in ice cream if, while in the freezer, it was in the metastable state.

At the present writing there are no data available from which to calculate the position of the metastable/labile boundary line in ice cream at the temperatures obtained in the freezer. The difficulty attending the experimental determination of this line is great, due to the formation of ice, to the possible influence of such mix ingredients as sugar, protein, fat and salts, and to the viscosity of the semi-frozen mass. It would appear from the work of Leighton and Peter (11) and from indirect evidence presented later in this paper, that in an ice cream mix of average composition it might be possible for the lactose to pass from the metastable to the labile state at about $-6.10$ to $-5.6°$ C. (21 to 22° F.). If conditions were such that the

lactose existed in the labile state during the freezing process, it seems likely that the sand defect would be very troublesome.

It is obvious that one of the chief factors which determine the metastable/labile boundary is the concentration of the lactose, or the percentage of milk-solids-not-fat which is of more significance to the ice cream manufacturer. When a mix contains more than 11.5 per cent milk-solids-not-fat or when the ratio of lactose to water is less than 1:12.5 the metastable/labile boundary may be found at a temperature higher than the minimum reached during the freezing process. In this case there will be a tendency for lactose to crystallize since agitation of supersaturated solutions in the labile state induces crystallization. On the other hand, in mixes containing a percentage of milk-solids-not-fat below the so-called "danger line" of 11 to 12 per cent; or having a lactose/water ratio greater than 1:12.5 the metastable/labile boundary line may be at a temperature below that reached in the freezer. In this event the tendency to crystallize will be small as the lactose always remains in the metastable state. This theory seems to provide an adequate explanation for the behavior of ice creams containing about 12 per cent milk-solids-not-fat which concentration is described by manufacturers as being the upper limit or "danger line."

*Influence of Freezer Operation on Lactose Crystallization*

As now manufactured the temperature of ice cream in the freezer rarely falls below −5.6° C. (22° F.) and is generally one to two degrees higher than this when it is drawn. If the assumption is made that the lactose in ice cream of average composition passes from the metastable to the labile state at a temperature of −6.1° to −5.6° C. (21 to 22° F.) it would be expected that the lactose would not crystallize in the freezer. Ice creams of varying composition have been frozen under a variety of conditions and no lactose crystals have ever been detected in the product while in the freezer. In extreme cases crystals have been detected in ice cream two days after freezing but generally at least several days are required to cause crystallization under adverse conditions. When the prediction is made that agitation of ice cream mixes containing lactose in the labile state induces crystallization, it must not be concluded that immediate precipitation of the lactose is implied, but rather that crystallization takes place one or more days sooner than normal, which may be a week or a month after freezing depending on the storage treatment the ice cream receives.

The metastable/labile boundary is not a sharp line. Lactose solutions in concentrations near this line do not always act as predicted from a theoretical point of view. For example, copious crystallization may take place slightly above this theoretical boundary in one case while in another crystals may not appear, although conditions indicate that the lactose is slightly below the boundary and in the labile area. Hence it may be argued that

in the case of ice cream in the freezer where the lactose may be near to the boundary crystallization may at some later date be influenced by the treatment the ice cream receives during the freezing process. It would seem, therefore, that the lower the temperature reached in the freezer the greater will be the tendency of the lactose to crystallize, as such a treatment places the lactose nearer to the labile area. It also follows that the longer the ice cream is agitated the greater will be the tendency for lactose to crystallize as agitation promotes nuclei formation and hastens crystallization.

In order to demonstrate this point a large batch of ice cream mix was divided into several portions, each of which was frozen to a different temperature. When the ice cream reached the desired temperature the circulation of the brine was so adjusted that that temperature was maintained for 10 minutes. Samples were drawn at three different intervals, one when the temperature was first attained, one when the ice cream had been whipped for five minutes at that temperature, and one after ten minutes. After hardening in the usual manner for two days the samples of ice cream were placed in an electric cabinet and held until lactose crystals could be detected microscopically. This experiment was conducted several times. Typical data from one series are presented in table 4.

TABLE 4

*Influence of temperature and length of time of whipping ice cream mix in the freezer on the rate of appearance of lactose crystals. Ice cream stored at −9.4° C. (15° F.) in electric cabinets*

| TEMPERATURE OF THE ICE CREAM IN FREEZER | | TIME THE ICE CREAM WAS REMOVED FROM FREEZER | | |
|---|---|---|---|---|
| | | Immediately on reaching desired temp. | After whipping for 5 minutes at desired temp. | After whipping for 10 minutes at desired temp. |
| °C. | °F. | Days to crystallize | Days to crystallize | Days to crystallize |
| −3.9 | 25 | * | 25 | 20 |
| −4.4 | 24 | 20 | 17 | 14 |
| −5.0 | 23 | 25 | 17 | 14 |
| −5.6 | 22 | 25 | 14 | 10 |
| −6.1 | 21 | 8 | 8 | 6 |

* No crystals present at 30 days.

Inspection of table 4 shows that the treatment given the ice cream in the freezer had an influence on lactose crystal formation. In general, the longer the ice cream was in the freezer and the lower the temperature, the sooner lactose crystallized. It will also be noted that lactose crystallized comparatively rapidly in the lot which was agitated at −6.1° C. (21° F.). This lends support to the theory that the metastable/labile boundary line may exist at or near this temperature.

*The Influence of the Rate of Hardening on Lactose Crystallization*

The temperature of ice cream when drawn from the freezer ranges from about −5.5° to −4.4° C. (22 to 24° F.). When the product is placed in the hardening room the temperature decreases to −18° C. (0° F.) or less. The time required to reach this temperature depends upon such factors as the amount of water frozen into ice before the ice cream leaves the freezer, size of containers, container material, method of stacking cans or packages, temperature of the hardening room, and circulation of air.

Ice cream manufacturers have found through experience that rapid hardening of ice cream is desirable. Quick hardened ice cream is less subject to the sand defect according to Bayer (16). In an experiment designed to show the influence of the rate of hardening on lactose crystallization three cans of ice cream filled from the freezer at the same temperature were cooled to −18° C. (0° F.) by three different methods. The bulb of the thermometer in all cases was placed two inches below the surface of the ice cream in the center of the can. One can was cooled rapidly by placing the can in an alcohol bath at −32° C. (−25° F.) until the temperature of the ice cream had reached −18° C. (0° F.). This required about one hour. Another can was cooled in the usual manner, by placing on the floor of the hardening room. The temperature was −20° C. (−4° F.). The temperature of the ice cream reached −18° C. (0° F.) in about 15 hours. The third can was cooled slowly by placing it in a closed corrugated cardboard box in the hardening room, the air in the box acting as an insulator. It required about 28 hours to cool the ice cream to the desired temperature of −18° C. (0° F.).

The three cans were placed in cabinets at −9.4° C. (15° F.) after one day at −18° C. (0° F.). The ice cream was examined weekly for lactose crystallization. The results of this experiment, given in table 5 show that the lactose crystallization was delayed by rapid hardening.

TABLE 5

*The influence of rate of hardening of ice cream on lactose crystallization*

| METHOD OF HARDENING | HOURS TO REACH −17.8° C. (0° F.) | DAYS TO FIRST LACTOSE CRYSTALS | |
|---|---|---|---|
| | | Experiment 1 | Experiment 2 |
| Quick | 1 | 51 | 32 |
| Medium | 15 | 44 | 25 |
| Slow | 28 | 37 | 19 |

Rapid hardening is to be desired from a theoretical point of view. It has been shown elsewhere that diffusion of the unfrozen portion is markedly reduced in ice cream at low temperatures. Since diffusion is necessary for crystal formation and growth, rapid hardening delays crystallization. In

like manner rapid attainment of low temperatures is desired as the rate of conversion of beta to alpha is decreased under such conditions.

### *The Influence of the Method of Packaging Ice Cream on Lactose Crystallization*

During a study of the influence of the method of packaging ice cream on lactose crystallization three methods of packaging were considered, namely: bulk ice cream drawn directly into cans from the freezer; quart boxes filled from the freezer; and quart boxes packed by hand from bulk goods.

Ice cream was frozen in the regular manner and drawn into five-gallon cans at 90% overrun. When filling the can a sample was also drawn into a cardboard quart pail. The samples were hardened in the usual manner and stored at – 18° C. (0° F.) for one week. At this period there were no lactose crystals visible under the microscope. Ice cream was then dipped from the five-gallon can and packed with a spoon into a quart ice cream pail. A temperature rise of about 5° C. (9° F.) was noted. The sample of ice cream which had been drawn directly into the pail from the freezer was then subjected to the same temperature rise, namely 5° C. (9° F.) for approximately the same length of time. The samples were returned to the hardening room for one day and then placed in an electric cabinet having a temperature range of – 12° to – 11° C. (10 to 12° F.). The ice creams were examined from time to time for lactose crystallization. Three series of samples were observed in this experiment. The observations are recorded in table 6.

TABLE 6

*Rate of lactose crystallization in bulk, hand packaged and freezer drawn ice cream*

| PERCENTAGE COMPOSITION OF MIX | | | TREATMENT GIVEN ICE CREAM | TIME TO LACTOSE CRYSTALLIZATION DAYS |
|---|---|---|---|---|
| Fat | Milk solids-not-fat | Sugar | | |
| 12 | 10 | 15 | Freezer drawn bulk | 27 (trace) |
| | | | Packaged by hand | 11 |
| | | | Freezer drawn pckg. | 18 |
| 14 | 11 | 15 | Freezer drawn bulk | 18 |
| | | | Packaged by hand | 12 |
| | | | Freezer drawn pckg. | 14 |
| 12 | 10 | 15 | Freezer drawn bulk | none at 38 |
| | | | Packaged by hand | 11 |
| | | | Freezer drawn pckg. | 23 |

Inspection of table 6 shows that the ice cream which had been packed by hand into ice cream pails developed crystals more rapidly than bulk goods

or packages filled directly from the freezer. Lactose crystallization in the bulk goods was delayed longer than in goods packed directly at the freezer.

The probable explanation for these observations is that the bulk ice cream experienced less temperature fluctuation because of the comparatively large volume. The hand packaged ice cream crystallized more rapidly than the ice cream packaged directly from the freezer because the agitation and localized temperature fluctuation during packaging with a spoon created conditions which fostered subsequent lactose crystallization. It is also possible that a structure was created during the hardening period which if left undisturbed tends to prevent lactose crystallization by retarding movement of the metacryotic liquid. If the structure was partially destroyed or changed, such as probably resulted during the hand packaging procedure, lactose crystallization was promoted due to the greater movement of unfrozen liquid in the mass.

It would seem that in bulk goods which harden more slowly the lactose would crystallize more rapidly, since it has been shown elsewhere in this report that the rate of hardening has an influence on lactose crystallization. However, the temperature fluctuation which packaged ice cream must encounter more than offsets the more rapid hardening of the smaller packages. Packaged goods are often stacked close together to facilitate handling and to effect economy of storage space, which condition fosters slow hardening.

### *Development of Lactose Crystals in Ice Cream in the Container*

In general lactose crystals were detected organoleptically in ice cream in all parts of the mass at about the same time, although some special cases were observed in five-gallon cans from time to time, especially when examined microscopically. In one special case crystals first appeared in the center of the can. This was traced to slow hardening in the center, while the outer layers cooled rapidly. In another case slight but frequent temperature fluctuations were found to be the cause of ice cream becoming sandy on the top and sides before the center. Fluctuation of temperature was also responsible for the ice cream toward the top of certain containers of ice cream developing the sand defect before the ice cream lower down. In this case the cans were stored in an electric cabinet, the lids of which were more or less frequently removed. Uneven distribution of crystallized lactose in ice cream was the exception rather than the rule and for all practical purposes it may be assumed that lactose crystallizes simultaneously throughout the entire mass of ice cream in packages and in the bulk containers.

### *The Influence of Temperature Fluctuations on Lactose Crystallization in Ice Cream*

Many investigators have observed that ice cream which was allowed to soften and was then rehardened was more likely to exhibit the sand defect

than ice cream which was not subjected to such temperature fluctuations (3), (4), (12), (14), (19). Severe temperature fluctuations were recorded when ice cream was stored in salt and ice cabinets. The introduction of the electric cabinet corrected this undesirable condition to a marked degree so that at the present time ice cream is seldom subjected to temperature variations. However, the fact remains that sandiness as due to partial melting and subsequent freezing is still a factor and may result because of insufficiently refrigerated trucks, slow handling, poorly adjusted thermostats on cabinets, carelessness in leaving lids off cabinet sleeves and the like.

The term "heat shock" has been applied to the process of raising the temperature of ice cream and later lowering it again. The use of this term is popular with commercial men, but is somewhat misleading since the idea of a sudden and rapid temperature change is implied, which is not always the case. The term "temperature fluctuation" is to be preferred for this reason.

When lactose crystallizes in properly hardened and stored ice cream small rather than large crystals form as there is little movement of the unfrozen portion throughout the mass. Diffusion is necessary for the production of large crystals. Among other things an increase in temperature causes an increase in diffusion and a decrease in viscosity which condition fosters the formation of large crystals.

When the temperature of ice cream is raised some ice melts. If the ice cream contains crystallized lactose the crystals will tend to dissolve due to the dilution caused by the melted ice. Under such conditions the smaller crystals will dissolve more readily than the larger ones. When the temperature is again lowered, lactose will be precipitated on the existing nuclei, *i.e.*, the larger crystals which failed to dissolve at the higher temperature. Thus the larger lactose crystals grow at the expense of the smaller ones in ice cream which is subjected to temperature fluctuations.

It has been shown elsewhere that in ice cream at low temperatures the rate of change of beta to alpha is less rapid than the rate of precipitation of alpha and the former becomes, therefore, an important factor in the rate of lactose deposition. Since the rate of change is proportional to the temperature it follows that a rise in temperature will make more alpha available when conditions again favor crystallization.

No actual experimental work was conducted on the influence of temperature fluctuation as this factor is well known and has been demonstrated many times. One interesting observation was made, however, which is worthy of record here. Samples of the same ice cream were stored in two electric cabinets operated as follows: Cabinet No. 1. An electric cabinet controlled by means of the gas pressure thermostat used on most commercial installations. It was adjusted so that the sleeve temperature range

was about 2.4° C. (4° F.). The maximum temperature was −9.4° C. (15° F.) and the minimum −11.7° C. (11° F.). Cabinet No. 2. The temperature was maintained at −9.4° C. ± 0.28° (15° F. ± 0.5°) by means of an electrically operated thermostat and relay. The thermostat was activated by the temperature of the brine surrounding the sleeves.

It was found that crystallized lactose appeared in the ice cream stored in Cabinet No. 1 about 10 to 14 days before that in Cabinet No. 2. It will be noted that the average temperature in Cabinet No. 1 was 1.1° C. (2° F.) below that of Cabinet No. 2 and at no time did it exceed the constant temperature of −9.4° C. (15° F.) of unit No. 2, yet the lactose in the ice cream in this cabinet crystallized first. This is an interesting illustration of the influence of temperature fluctuation on the rate of lactose crystallization in ice cream.

*The Effect of Mechanical Shocks on Lactose Crystallization in Ice Cream*

Mechanical shock is a broad term used by ice cream manufacturers to denote any bumping, shaking or vibration to which the cans of ice cream may be subjected, as for example being dropped on the floor, shaken in a truck driven over poor roads, vibration in a cabinet, and the like. The literature contains statements which imply that ice cream which has been exposed to mechanical shocks is likely to exhibit the sand defect (12) (18).

The theory has been advanced that crystallization of the lactose in ice cream is promoted by sudden shocks or long continued vibration. Such treatment probably causes a sudden movement of the metacryotic liquid in the frozen mass, which may induce crystallization of the lactose or promote the growth of existing crystals. Experimental work has been conducted to show that sudden jars induce crystallization in pure solution but no actual experiments have been described in the literature, wherein the lactose in ice cream was made to crystallize prematurely by means of mechanical shocks.

Three 10-quart cans were filled with ice cream from the same freezer and hardened in the regular manner for two days. The cans were then transferred to an electric cabinet which was operating at −12° C. (10° F.). The cans were given the following treatments: One can was stored continuously in the cabinet. Another can was removed daily from the cabinet for a period of five to ten minutes and bounced or dropped on a concrete floor from the height of about one foot. The third can was removed daily from the cabinet for the same period of time as the second can. This was not subjected to the jarring procedure but was merely removed from the cabinet as a check or control sample.

Microscopic examination of the ice cream in the three cans showed that crystallized lactose appeared in all three at the same time, indicating that

the mechanical shocks given the ice cream under the conditions of this experiment had no influence on lactose crystallization.

Crystallization of substances from supersaturated solutions frequently results when the container holding the supersaturated mass is caused to vibrate. It was believed that the motor and compressor of electric cabinets might transmit to ice cream in cans sufficient vibratory motion to cause premature crystallization of the lactose.

In order to demonstrate whether or not vibration is carried from the motor and compressor to ice cream in cans within the sleeves vibration measurements were made with a device supplied by the Research Laboratories of the Frigidaire Corporation of Dayton, Ohio. It was found that the frames of the cabinets vibrated to the extent of 0.003 to 0.005 inches but that the cans within the sleeve exhibited vibrations of less than 0.001 inches. Cabinets of portable type which have the motor and compressor mounted on the same chassis as the cabinet, showed slightly more vibration than cabinets which were not directly connected with the refrigeration unit.

When duplicate samples of ice cream were stored in these cabinets, the lactose crystallized in all at the same time. Identical and constant temperatures were maintained in both cabinets by means of thermostats. During one trial the compressor and motor of the portable cabinet was bolted directly to the frame, the rubber supports having been removed. Even this abnormal condition failed to produced sufficient vibration to cause the premature development of crystallized lactose in the ice cream.

It appears that mechanical shocks of the types employed in this investigation cannot be considered as significant factors in promoting the formation of sand in ice cream.

### *Nuclei as a Factor in the Rate of Lactose Crystallization*

Crystallization can be induced in many supersaturated solutions by seeding with a few crystals of the dissolved substance or with other substances possessing certain characteristics. In general, substances which are relatively insoluble, are of crystalline structure or which have sharp pointed or angular particles, are the best seeding agents or nuclei.

To affect premature crystallization of lactose in ice cream by seeding with pulverized alpha lactose it was necessary to distribute the seed or nuclei throughout the entire mass. Placing of the seed in one part of the mass did not produce crystallization throughout the mass but did induce copious crystallization in the vicinity of the seed. This was determined by actual trial.

When insoluble particles of foreign substances were introduced into ice cream the lactose crystallized prematurely, as can be seen from table 7. The data was procured by examining stored samples of ice cream drawn from the same freezer, into each of which had been stirred one of the vari-

TABLE 7

*The influence of the presence of insoluble foreign material, as seed or nuclei, on the rate of lactose crystallization in ice cream*

| MATERIAL ADDED TO ICE CREAM | TIME TO FIRST APPEARANCE OF LACTOSE CRYSTALS | |
|---|---|---|
| | Cabinet 1 | Cabinet 3 |
| | *days* | *days* |
| Nothing | none at 28 | none at 28 |
| Asbestine | 21 | lost |
| Sand | 23 | 21 |
| Kaolin | 23 | 17 |
| Ground glass | 17 | 13 |
| Carborundrum | 23 | 17 |

ous materials listed in the table. The seeding agents were added at the rate of approximately 1 per cent.

### *The Influence of the Temperature of Pasteurization of the Mix on Rate of Lactose Crystallization in Ice Cream*

Lucas and Spitzer (4), investigating the influence of the temperature of pasteurization on the rate of sand development in ice cream, found that "sandiness was neither prevented nor affected so far as could be determined by high or low pasteurization temperatures." These workers varied the pasteurization temperatures from 62.8 to 73.9° C. (145 to 165° F.).

There are recorded in table 8 data produced in two series of experiments

TABLE 8

*The influence of the temperature of pasteurization on the rate of lactose crystallization in ice cream*

| TEMPERATURE OF PASTEURIZATION | | TIME OF PASTEURIZATION | TIME TO FIRST LACTOSE CRYSTALS | |
|---|---|---|---|---|
| | | | Cabinet 1 | Cabinet 3 |
| °C. | °F. | *min.* | *days* | *days* |
| | | *Series A* | | |
| 62.8 | 145 | 30 | 43 | 51 |
| 82.2 | 180 | 10 | 63 | none at 69 |
| | | *Series B* | | |
| 62.8 | 145 | 30 | 8 | 11 |
| 71.1 | 160 | 30 | 8 | 14 |
| 79.4 | 175 | 15 | none at 36 | none at 47 |

designed to show the effect of the temperature of pasteurization on lactose crystallization in ice cream. In Series A the composition of the mix was 12 per cent fat, 10 per cent milk-solids-not-fat, and 15 per cent sugar. In

Series B the composition was 13 per cent fat, 11 per cent milk-solids-not-fat and 15 per cent sugar. It will be noted that lactose crystallization was materially retarded by pasteurizing at 79.4 to 82.2° C. (175° to 180° F.). Little or no benefit was gained by pasteurization at 71.1° C. (160° F.) was compared to 62.8° C. (145° F.).

In Series A the homogenization process was conducted at the pasteurization temperature, while in Series B all mixes were homogenized at 62.8° C. (145° F.). From the similarity of the results obtained in the two series it appears that it is the pasteurization temperature and not the homogenization temperature which influenced the rate of crystal development in these studies.

### *The Influence of Nut Meats on the Crystallization of Lactose in Ice Cream*

It is generally agreed that ice cream containing nut meats develops sand more frequently than other flavors. This peculiarity has drawn the attention of Reid (12) and Dahle (19) who have probably done the most work on this subject. Reid believes that the nut meats act as nuclei which induce premature lactose crystallization in ice cream.

Reid showed that the sand defect in nut ice cream was delayed when the nut meats were washed with warm water, autoclaved under steam pressure or soaked in a gelatin solution prior to use in ice cream. Dahle (19) soaked nut meats in a sugar syrup before using in ice cream, and delayed sandiness. Since all of these practices resulted in the nuts taking up moisture before being placed in the ice cream, it may be argued that unsoaked nut meats when placed in the ice cream imbibe water, thereby causing an increase in the concentration of the lactose in the unfrozen portion of the ice cream adjacent to the nut meat particle. The increase may be of sufficient magnitude to cause premature crystallization of lactose.

It was observed that more and larger lactose crystals were found close to the nut meats particles than in the ice cream between the particles. This lends support to the hypothesis advanced above. That this explanation based on water absorption adequately accounts for the premature crystallization of lactose in nut ice cream was shown by some experimental work.

In order to demonstrate the water imbibing property of nut meats such as are used by the ice cream industry, samples of five kinds of nuts were procured and chopped into particles about the size of wheat kernels. A known weight of each kind was placed in three times their weight of water at 4° C. (40° F.). After 18 hours the nuts were again weighed. The data collected in this experiment are presented in table 9.

The above experiment was repeated, using a 10 per cent lactose solution instead of water to demonstrate that the nuts did not remove lactose from solution. The solutions were analyzed for lactose before and after soaking

TABLE 9
*The water-absorbing power of chopped nut meats*

| KIND OF NUT | WT. BEFORE TREATMENT | WT. AFTER SOAKING IN WATER FOR 18 HOURS | AMT. OF WATER ABSORBED BY NUTS |
|---|---|---|---|
| | *gms.* | *gms.* | *per cent* |
| Black walnut | 45 | 65 | 44 |
| Manchurian walnut | 45 | 70 | 57 |
| Domestic walnut | 45 | 65 | 44 |
| Almond | 45 | 75 | 66 |
| Burnt Almond | 45 | 65 | 44 |

the nut meats. In this work 12.5 gms. chopped nuts were added to 30 cc. of lactose solution for 15 hours. The data are given in table 10. These data clearly show that nut meats are capable of removing water from a lactose solution, thereby causing an increase in lactose concentration.

Experimental work was next conducted on ice cream. Both dry and soaked nut meats were added to ice cream at the rate of 45 gms. of nuts per quart of ice cream. This was done by first placing the prepared nuts in empty ice cream pails. These were then filled with ice cream direct from the freezer. The nuts and ice cream were then thoroughly mixed together by stirring with a spoon. The samples were hardened for 24 hours—at

TABLE 10
*The water-absorbing power of chopped nut meats in a 9.6 per cent lactose solution*

| KIND OF NUT | PER CENT OF LACTOSE | | PER CENT DIFFERENCE |
|---|---|---|---|
| | Before soaking | After soaking | |
| Black walnut | 9.60 | 10.92 | 1.32 |
| Manchurian walnut | 9.60 | 10.96 | 1.36 |
| Domestic walnut | 9.60 | 10.66 | 1.06 |
| Almond | 9.60 | 11.64 | 2.04 |
| Burnt almond | 9.60 | 9.92 | 0.32 |

−18° C. (0° F.), placed in a cabinet at −12° C. (10° F.) and examined frequently for lactose crystallization. The results of this experiment are given in table 11. There are also included in this table data collected on samples of nut ice cream made in the regular manner, *i.e.*, chopped nut meats added to the freezer. The nuts were added at the rate of 5 per cent by weight. Inspection of table 11 shows that lactose crystals appeared in the ice cream containing the dry or unsoaked nuts sooner than the ice cream made with the soaked nut meats. The time to lactose crystallization as

TABLE 11

*The influence of nut meats on the rate of lactose crystallization in ice cream*

| KIND OF NUT | TREATMENT | WHEN ADDED TO ICE CREAM | TIME TO APPEARANCE OF LACTOSE CRYSTALS |
|---|---|---|---|
| | | | *days* |
| Black walnut | none | after drawing | 7 |
| | soaked | " " | 17 |
| | none | in freezer | 3 |
| Manchurian walnut | none | after drawing | 7 |
| | soaked | " " | 15 |
| | none | in freezer | 3 |
| Domestic walnut | none | after drawing | 7 |
| | soaked | " " | 17 |
| | none | in freezer | 3 |
| Almond | none | after drawing | 3 |
| | soaked | " " | 17 |
| | none | in freezer | 3 |
| Burnt almond | none | after drawing | 7 |
| | soaked | " " | 15 |
| | none | in freezer | 3 |
| None | none | control | 20 |

given in this table was taken when lactose crystals were first observed. In every case crystals appeared adjacent to the nut meats before their presence was noted throughout the entire mass.

It was also possible to show a difference in lactose content in ice creams between samples frozen with and without nut meats, after 2 days in the hardening room. In this case the ice cream was carefully melted and the mix analyzed for lactose.

### *The Influence of the Degree of Fat Globule Clumping on Lactose Crystallization in Ice Cream*

Ice cream mixes in which the fat globules are clumped are more viscous than mixes which contain a minimum amount of fat globule clumping. It would seem that in mixes of high viscosity the movement of the metacryotic liquid in the frozen ice cream would be less than in ice cream made from mixes of low viscosity. Since the rate of lactose crystallization in ice cream is dependent in good part on the rate of diffusion of the unfrozen constituents, it may be predicted that lactose crystals will develop less readily in ice cream, the fat globules of which are clumped, than in ice cream which contains the fat in a well-dispersed condition. Experimental work was undertaken to demonstrate this point.

An ice cream mix containing 13 per cent fat, 11 per cent milk-solids-not-fat, and 15 per cent sugar was divided into two parts. One batch was pasteurized at 65.5° C. (150° F.) for 30 minutes, then homogenized at that

temperature through a two-stage valve, using 2500 lbs. pressure on the first valve and 1000 lbs. on the second. A microscopic examination of this mix showed that there was practically no fat globule clumping. The other batch was treated to induce fat globule clumping, by homogenizing at a lower temperature and with one valve. In this case the pasteurization was conducted the same as above but the mix was cooled to 48.9° C. (120° F.) before homogenization with 2500 lbs. pressure on the one valve. Examination of this mix showed extensive fat globule clumping.

The two mixes were then frozen and samples placed in electric cabinets after being hardened. The ice cream was examined from time to time for lactose crystallization. The data collected during one such experiment are presented in table 12.

TABLE 12

*The influence of fat globule clumping as caused by homogenization conditions on the rate of lactose crystallization in ice cream*

| FAT GLOBULES | VISCOSITY | TIME OF APPEARANCE OF LACTOSE CRYSTALLIZATION | |
|---|---|---|---|
| | | Cabinet 1 | Cabinet 2 |
| | *sec.* | *days* | *days* |
| Not clumped | 16½ | 11 | 17 |
| Clumped | 161 | 31 | 31[1] |

[1] Sample discarded on this day—no crystallization.

Inspection of table 12 shows that lactose crystals appeared first in the ice cream which was treated to prevent fat globule clumping. It will also be observed that this mix was of low viscosity. Crystallization was delayed by producing a mix of high viscosity by adjusting the homogenization procedure to induce fat globule clumping.

Several investigators have shown the importance of various salts on the properties of ice cream, and have presented data which indicate that salts have a marked influence on fat globule clumping. The viscosity of ice cream mix is due in part to the extent of clumping. As far as could be ascertained, no work has been published on the effect of salts on the rate of crystallization of lactose in ice cream.

A quantity of ice cream mix was divided into a number of batches of equal weight. There was added to each batch prior to pasteurization and homogenization, a known quantity of one of several salts dissolved in a small quantity of water. The mixes were all processed and frozen under the same conditions. Observations on the rate of lactose crystal development were recorded. These are summarized in table 13.

Table 13 shows that the appearance of crystallized lactose in ice cream was postponed by the addition of the divalent elements, calcium and mag-

TABLE 13

*The influence of various salts on the rate of lactose crystallization in ice cream*

| KIND OF SALT ADDED | SALT CONCENTRATION | FAT GLOBULE CLUMPING | ACIDITY | TIME TO LACTOSE CRYSTALLIZATION | |
|---|---|---|---|---|---|
| | | | | Experiment 1 | Experiment 2 |
| | *per cent* | | *per cent* | *days* | *days* |
| None | | + | 0.22 | 25 | 18 |
| Calcium lactate | 0.1 | +++ | 0.24 | 32 | 32 |
| Calcium lactate | 0.05 | +++ | 0.23 | 36[1] | 34[1] |
| Magnesium lactate | 0.1 | +++ | 0.23 | 39[1] | 34[1] |
| Magnesium lactate | 0.05 | +++ | 0.22 | 39[1] | 32 |
| Calcium hydroxide | 0.1 | ++ | 0.16 | 25 | 22 |
| Calcium chloride | 0.05 | +++ | 0.22 | 39[1] | 29 |
| Sodium citrate | 0.1 | + | 0.22 | 23 | 20 |
| Sodium bicarbonate | 0.2 | + | 0.20 | 25 | 18 |

[1] Samples discarded on day indicated; no crystals present.

nesium, whereas sodium salts had no effect. It will be noted that the calcium and magnesium ions increased the degree of fat clumping in the mix. This action was very pronounced in some cases, especially when 0.2 per cent of the salt was added to the mix prior to homogenization. The increase in viscosity due to fat globule clumping probably decreased the rate of diffusion of the metacryotic liquid, thereby retarding crystallization of the lactose. The action of these salts cannot be attributed to the change in acidity, as it has been shown by several workers (4) (14) (21) that the acidity has no significant effect on the rate of lactose crystallization.

## SUMMARY

A brief review of the theories concerned with the crystallization of lactose in ice cream has been presented, including a discussion of the properties of supersaturated lactose solutions, isomeric forms of lactose, and the rate of diffusion in ice cream at low temperatures.

Four methods of detecting crystallized lactose in ice cream were studied. The microscopic method has been given in detail because of its employment in this research.

The following factors and their influence on lactose crystallization in ice cream were discussed: Composition of the mix, freezer operation, rate of hardening, method of packaging, development of crystals throughout the mass of ice cream, temperature fluctuations, mechanical shocks, presence of seed or nuclei, mix pasteurization temperature, presence of nut meats and extent of fat globule clumping.

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# A STUDY OF THE PHOSPHORUS REQUIREMENT OF DAIRY CATTLE

## I. Phosphorus Requirement for Growth and Reproduction from Three Months of Age to First Calving[1]

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In previous reports from this Station (1) (2) it was shown that dairy cattle fed liberally on palatable hay along with a grain mixture containing a high phosphorus protein concentrate received sufficient calcium and phosphorus for the production of at least 10,000 pounds of milk a year. However, Huffman and Taylor (3) in 1924 while studying depraved appetite of cattle under field conditions observed that phosphorus deficiency occurred among milking cows which received legume roughage supplemented with the cereal grains. This observation suggested that possibly a home grown ration consisting of low phosphorus alfalfa hay, corn silage and corn might be used as a basis for studying the phosphorus requirement of dairy cattle, since such a ration furnished sufficient protein and energy for growth in young animals and for the production of about 40 pounds of milk per day in lactating cows (4).

Fourteen grade Holstein heifers are being used in this investigation. These animals were started on experiment at 90 days of age and will be continued through three lactation periods to determine the effect of a low phosphorus ration on growth, health, reproduction, and milk production when alfalfa furnishes the principal source of protein. The present report presents the data to first calving.

### LITERATURE CITED

### *Symptoms of Phosphorus Deficiency*

An extensive review of the literature giving the symptoms of phosphorus deficiency has been given by Eckles, Becker and Palmer (5), Henderson and Weakley (6), Scott (7), and Orr (8). Consequently, only the most outstanding symptoms of phosphorus deficiency will be reviewed in this paper.

Henry (9) described the typical symptoms of phosphorus deficiency among cattle in Australia as stiffness in the hind quarters, swelling of the

Received for publication July 28, 1932.

[1] Journal Article No. 108 (N. S.) from the Michigan Agricultural Experiment Station.

joints, a harsh dry coat, dull eyes, unthrifty general appearance, perverted appetite, leg and rib bones easily broken (soft bones in some, brittle in others) and decreased milk yield. Welch (10) emphasized depraved appetite. He also attributed the high abortion rate on affected farms to phosphorus deficiency. Tuff (11) referred to paralysis as a common occurrence among animals suffering from phosphorus deficiency.

Theiler and associates (12) observed depraved appetite for bones as the principal symptom of phosphorus deficiency. They also found that a lack of appetite for normal food was associated with phosphorus deficiency, and that cattle under such conditions did not make good use of their food for body gains.

*Growth*

Kellner (13) computed the phosphorus retention of growing calves as 8.3 grams of phosphorus per day during the first year. He stated that the feed should contain from two to three times as much as required by the body or from 16.6 to 24.9 grams of phosphorus per day.

Lawes and Gilbert (14) reported that a calf contained 1.2 per cent calcium and 0.67 per cent phosphorus. Armsby (15) computed from Lawes and Gilbert's analysis of the ash of the entire body of farm animals that during the first year, cattle retain 8.14 grams of phosphorus per day. This figure agrees with Kellner's.

Henderson and Weakley (6) studied the effect of different levels of phosphorus in the ration on the growth of dairy heifers. These workers found that a ration containing less than 0.20 per cent phosphorus gave rise to bones which were low in ash and therefore low in calcium and phosphorus.

Theiler, Green and du Toit (16) in studying the mineral requirements in cattle found that a ration supplying 2.23 grams of phosphorus and 4.99 grams of calcium per day was deficient. Animals from 12 to 18 months of age withstood this level of mineral intake for three to six months. They then showed symptoms of mineral deficiency disease.

These investigators also fed two heifers the basal ration supplemented with 25 grams of bone meal daily which increased the phosphorus intake to about five grams per day which resulted in fair growth. When 100 grams of bone meal were added to the basal ration and the phosphorus increased to about 13 grams daily good growth and reproduction resulted. Also the addition of two pounds of wheat bran daily to the basal ration resulted in fair growth in body weight even though the calcium intake was only about six grams and the phosphorus about 11 grams per day.

Theiler and co-workers (17) stated that in any given diet it is the percentage of phosphorus in relation to the total feeding value which is of importance in determining osteophagia. They also found that with cattle grazing on the veld a daily consumption of about 11.79 grams of phosphorus

was regarded as somewhere about the point at which osteophagia could develop or disappear in grazing cattle of 1000 pounds live weight. They stated that for old oxen the intake of 11.79 grams of phosphorus per 1000 pounds was probably more than enough to prevent bone chewing, while for young cattle or milking cows this intake was certainly too low. These workers reported a marked increase in rate of growth by the addition of bone meal to phosphorus deficient rations.

### *Calcium-Phosphorus Ratio*

The calcium-phosphorus ratio in the diet has been the subject of many investigations. McCollum and co-workers (18) showed the effect of varying the ratio of calcium to phosphorus in the diet on the histological picture of the bones.

McCollum, Simmonds, Shipley and Park (19) observed that the ratio between calcium and phosphorus in the diet may, within certain limits, be of greater significance to the welfare of the animal than the absolute amounts of these substances contained in the diet. Bethke and co-workers (20) working with chickens found that the optimum or nearly optimum calcium-phosphorus ratio lies between 3:1 and 4:1.

Haag and Palmer (21) after studying the effects of variation in the proportions of calcium, magnesium and phosphorus in the diet of rats concluded that the general trend of their results left no doubt that a more or less balanced condition of calcium, magnesium, and phosphorus salts in the ration was essential to normal growth and functioning.

Meigs and co-workers (22), using four lactating cows, concluded that phosphorus assimilation may be interfered with by an excess of calcium in the ration and that two parts or more by weight of calcium to one of phosphorus constituted an excess. Turner, Harding and Hartman (23) working with two cows found a better assimilation of calcium from alfalfa hay than from clover. Their metabolism data suggested a better assimilation of calcium and phosphorus when the value of the calcium-phosphorus ratio was 1:1.25 than when it was 1:2.5.

Scott (7) found that the proportion of calcium to phosphorus in the food supply was a factor in regulating the amount of phosphorus retained by the growing animal. He drew attention to the fact that on a tricalciumphosphate basis the legumes have a large excess of calcium over that required for chemical combination with phosphorus. He also found that wild grasses from areas free from phosphorus deficiency showed an excess of phosphorus over calcium, but that corresponding samples from areas affected with phosphorus deficiency showed an excess of calcium.

Recent work of Haag, Jones and Brandt (24) indicates that a calcium-phosphorus ratio of 10.5 is no more detrimental than one of 7.6.

### *Reproduction*

Tuff (11) observed in deficient areas in Norway that in seasons of low rainfall reproductive troubles increased. Eckles, Becker and Palmer (5) found that in the phosphorus deficient areas in western Minnesota considerable troubles were encountered with breeding. On many farms some cows produced but one calf in two years. Heifers did not show signs of estrum until two years of age. The average calf crop in the most severely affected districts after a dry season was estimated at less than 50 per cent of the normal.

Theiler and associates (16), after studying the breeding of cows on phosphorus deficient pasture for two years, found that in a group not receiving a phosphorus supplement on pasture only 51 per cent of the cows calved normally, as compared with 80 per cent in a group receiving bone meal. Calves from cows receiving bone meal and fed bone meal after weaning were far superior at one year of age to those from control cows which did not receive bone meal after weaning.

Orr (25) stated that in areas where there was a general poverty of minerals the rate of growth of the young animal was slow and that the milk yield of cows was low.

Welch (10) attributed high abortion rate on affected ranches as being due to a lack of phosphorus.

### *Relation of Alfalfa to Phosphorus Deficiency*

Several investigators have reviewed the work showing the calcium and phosphorus content of grasses and hay from affected and unaffected regions —Eckles and associates (5), Hart (26), and Scott (7).

In this report only the composition of alfalfa and its relation to phosphorus deficiency will be considered, due to the greater prevalence of phosphorus deficiency in Michigan among animals fed alfalfa hay as the principal source of protein. The phosphorus-nitrogen ratio is probably an important factor responsible for this phenomenon. We are not interested in the phosphorus content of timothy hay and the other low protein roughages because such roughages require protein concentrates rich in phosphorus as supplements in order to meet the need for growth and milk production of dairy cattle. In order to show the relation of alfalfa to phosphorus deficiency the following table has been prepared from a limited number of analyses showing the calcium, phosphorus, and nitrogen content of alfalfa from various sections of the United States.

### *Relation of Vitamin D to Phosphorus Requirement of Cattle*

Investigations at the Pennsylvania Agricultural Experiment Station (42) and recent work at the University of Wisconsin (43) and the Michi-

TABLE 1

*Showing calcium, phosphorus, and nitrogen content of alfalfa hay from different sections of the United States as reported in the literature*

| LOCATION | | Ca | P | N | Ca/P | N/P |
|---|---|---|---|---|---|---|
| Minnesota (5) | P-deficient | 1.89 | 0.193 | | 9.8 | |
| " | unaffected | 1.76 | 0.257 | | 6.8 | |
| " (27) | P-deficient | | 0.122–0.258 | | | |
| " | unaffected | | 0.201–0.306 | | | |
| " | no treatment | | 0.363 | 4.51 | | 12.4 |
| " | P treatment | | 0.485 | 4.59 | | 9.5 |
| Montana, 1925 (28) | P-deficient | 1.72 | 0.109 | | 15.8 | |
| " " | unaffected | | 0.181 | | | |
| " 1927 | unaffected | | 0.164 | | | |
| " " | 1st cutting, unfertilized | | 0.161 | 2.79 | | 17.3 |
| " " | 1st cutting, fertilized | | 0.203 | 3.00 | | 14.8 |
| " " | 2nd cutting, unfertilized | | 0.179 | 3.25 | | 18.1 |
| " " | 2nd cutting, fertilized | | 0.221 | 3.47 | | 15.7 |
| Michigan (29) | | 1.60 | 0.178 | 2.42 | 9.0 | 13.6 |
| Kentucky, 1928 (30) | Mayfield, West Ky. | 0.90 | 0.250 | 2.38 | 3.6 | 9.5 |
| " 1930 | Campbellsville | 2.34 | 0.156 | 2.72 | 15.0 | 17.4 |
| " " | Princeton | 2.04 | 0.165 | 2.81 | 12.4 | 17.0 |
| Oregon (24) | | 1.16 | 0.153 | 2.06 | 7.6 | 13.5 |
| Wisconsin (26) | P-deficient | | 0.143 | | | |
| " (31) | | 3.09 | 0.270 | | 11.4 | |
| " (32) | | 1.83 | 0.226 | | 8.1 | |
| " (33) | 2nd cutting—cured under cap | 1.30 | 0.320 | | 4.1 | |
| " | green air dried | 1.02–1.47 | 0.25–0.32 | | 4.1–4.6 | |
| Nevada (34) | not irrigated | 1.67 | 0.175 | 2.32 | 9.5 | 13.5 |
| " | irrigated | 1.27 | 0.220 | 2.50 | 5.8 | 11.4 |
| Maryland (22) | good alfalfa | 2.266 | 0.169 | 2.095 | 13.4 | 12.4 |
| " | poor alfalfa | 1.925 | 0.172 | 2.004 | 11.2 | 11.6 |
| " | | 0.943 | 0.326 | 2.45 | 2.9 | 7.5 |
| Louisiana (35) | 1st cutting | | 0.267 | 2.49 | | 9.3 |
| " | 2nd cutting | | 0.297 | 2.57 | | 8.7 |
| " | 3rd cutting | | 0.315 | 3.11 | | 9.9 |
| South Carolina (36) | Peruvian | 1.93 | 0.246 | | 7.9 | |
| " " | Kansas | 1.80 | 0.272 | | 6.6 | |
| " " | Dakota | 1.49 | 0.277 | | 5.4 | |
| Ohio (37) | 1st cutting | 1.685 | 0.280 | | 6.0 | |
| " | 2nd cutting | 1.166 | 0.230 | | 5.1 | |
| " | fertilized | 1.512 | 0.290 | | 5.2 | |
| " | unfertilized | 1.649 | 0.273 | | 6.0 | |
| " (38) | | 1.202 | 0.172 | 2.334 | 7.0 | 13.5 |
| " (39) | | 1.156 | 0.206 | 2.342 | 5.6 | 11.4 |
| " (40) | | 1.130 | 0.238 | | 4.7 | |
| Washington (41) | ¼ bloom | 1.259 | 0.176 | | 7.2 | |
| " | ½ bloom | 1.408 | 0.160 | | 8.8 | |
| " | ¾ bloom | 1.311 | 0.129 | | 10.2 | |

gan Agricultural Experiment Station (44) indicates that the vitamin D requirement of calves is greater than has been believed heretofore. Results at the Michigan Station show that calves fed a rachitic ration and kept

away from sunshine develop rickets, while calves on the same basal ration and exposed to sunshine do not develop rickets. The vitamin D content of the ration and the exposure of the animals to sunlight probably affect the phosphorus requirement.

According to Steenbock, Hart, Elvehjem and Kletzien (45) the antirachitic potency of hay can be increased by exposure to ultra-violet light. Recent results at the Michigan Station (44) show that hay cured in the sun contains sufficient vitamin D to protect calves from rickets when fed a ration low in this vitamin. The importance of the vitamin D content of sun-cured hay as well as the value of sunshine must be taken into consideration in determining the calcium and phosphorus requirements of dairy cattle. According to Eckles and associates (5) the usual period of most severe depraved appetite is from February to May.

### *Blood Picture in Phosphorus Deficiency*

Meigs, Blatherwick and Cary (46) have reported variations in inorganic blood phosphate due to (a) changes in the phosphorus content of the ration, (b) increasing age, (c) pregnancy and (d) lactation. They found the inorganic blood phosphorus to be highly variable, not only from animal to animal, but also in the same animal examined at different times. We (47) have previously indicated that the amount of phosphorus in the diet of dairy cattle has a marked influence on the inorganic blood phosphate. Theiler, Green and du Toit (16) were the first to produce an experimental "Styfsiekte" in young heifers by feeding a ration very low in phosphorus. They reported 5.2 mg. per cent of inorganic phosphorus in the blood as normal while their experimental animals had blood values as low as 1.3 mg. per cent. Palmer and Eckles (48) have reported low inorganic phosphate values (mean value 1.85 mg. per cent) of animals raised in a phosphorus deficient region. Malan, Green and du Toit (49) have shown that heifers raised on phosphorus deficient pastures up to two years of age have had an average blood phosphate value of 2.3 mg. per cent, while cows have had values as low as 1.0 mg. per cent. The results from the investigation of Henderson and Weakley (6) show that as soon as the phosphorus in the feed is lowered, the inorganic blood phosphorus is decreased. They have shown changes from 8.15 and 7.93 mg. per cent to 4.86 and 4.35 mg. per cent, respectively, in a period of four days.

## EXPERIMENTAL

Two groups of seven grade Holstein heifers each were placed on experiment at 90 days of age to determine the phosphorus requirement of dairy cattle for growth, reproduction, milk production, and health over a period of five years when alfalfa furnished the principal source of protein. The heifers in lot I, D3, D5, D7, D9, D11, D13, and D15, were fed the basal

ration low in phosphorus. Those in lot II, D2, D4, D6, D8, D10, D12, and D14, were fed the basal ration supplemented with steamed bone meal.

The animals in both groups were all sired by the same bull and were from cows which were closely related. Heifers D3 in lot I and D14 in lot II were full sisters. Also, D5 in lot I and D12 in lot II were full sisters.

The animals were divided into the two groups according to the milk production of their dams rather than on the basis of body weight since we were more interested in the phosphorus requirement for milk production than the phosphorus requirement for growth. This accounts for the slight weight advantage given the heifers in lot II at the beginning of the experiment.

*History of Calves from Birth to 90 Days of Age*

The calves used in this investigation were fed whole milk from birth to about eight weeks of age and skimmilk after eight weeks of age. A mixture of equal parts of yellow corn and oats by weight and alfalfa hay were fed *ad lib.* from birth to 90 days of age. Ten cubic centimeters of cod-liver oil per day were also fed during this period. The calves were turned into a dry lot for exercise and sunshine daily when the weather would permit.

*Rations*

Lot I was fed the basal ration low in phosphorus which consisted of alfalfa hay, corn silage, yellow corn, and salt. Lot II was fed the same basal ration supplemented with special steamed bone meal. Both lots were fed skimmilk to about 110 days of age.

The heifers in lot I were fed a ration containing about 0.2 per cent of phosphorus and those in lot II were fed about 0.41 per cent of phosphorus on the dry matter basis. Lot II were fed an optimum phosphorus ration according to McCollum (50).

The animals in both groups were turned into a dry lot daily for exercise and sunshine. During good weather the calves remained in the lot about seven hours per day during the spring, summer, and fall. During the winter this time was reduced.

Water intake was measured by means of water meters after the heifers were placed in stanchions at about one year of age. Before this time they were watered from a tank in the exercise lot and the water consumption estimated. The phosphorus intake of the heifers in lot I varied from 5.7 to 9.9 grams and in lot II it varied from 10.3 to 19.7 grams daily during the first 18 months.

*Feeds Used*

Sun-cured alfalfa hay with a phosphorus content of less than 0.2 per cent phosphorus was fed to the animals in both lots. An attempt was made to obtain alfalfa hay containing at least 15 per cent protein. The alfalfa

hay was ground before being fed in order to facilitate weighing and to insure even consumption. No. 2 ground yellow corn was used. All feeds fed were weighed and recorded. Any refused feed was weighed back. Total feed consumption from three to 18 months is shown in table 2. Average feed consumption is shown by three-month periods in table 3. The feeds were analyzed for calcium, phosphorus, and protein from time to time.

### *Growth*

The animals were weighed at 10-day periods to 15 months of age. After 15 months of age they were weighed on three consecutive days at the beginning of each month and an average of the weights was used. The animals were weighed in the morning before feeding. Growth in height at withers was measured each month.

The gain in body weight, height at withers, and nutrients consumed by individual animals from three months to 18 months are shown in table 2. Since the body weights and heights at withers of the two groups varied slightly at 90 days of age the effect of the low phosphorus ration on growth is best shown in the above table. Growth in weight was not considered after 18 months because of the effect of gestation.

The average growth of the two lots from birth to 24 months of age by three month periods is shown in table 3.

### *Reproduction*

The animals were negative to *Brucella abortus* infection. No reproductive disturbances due to a lack of phosphorus in the ration were encountered during the first gestation period.

### *Appetite*

*Pica or depraved appetite:* The animals in both lots were tested for bone chewing from time to time to first calving. They were also observed for wood chewing, hair chewing, or for any other abnormal cravings.

D3 and D15 in lot I which received the ration low in phosphorus manifested the most pronounced depraved appetite for bones and wood from 90 days to first calving. However, this condition, although manifested, was not so marked in heifers D5, D7, D9, D11 and D13 of lot I.

The animals in lot II which received the basal ration supplemented with bone meal showed a slight amount of wood chewing soon after being placed on experiment. This condition soon cleared up and probably was not due to the phosphorus level in the ration.

*Anorexia or lack of appetite:* The animals in lot I (low phosphorus group) had poor appetite for roughage when compared with those in lot II (high phosphorus group). This difference is shown in table 2.

TABLE 2

*Showing gain in weight and height, and food consumption of heifers in lots I and II from 3 to 18 months of age*

*Lot I. Basal ration low in phosphorus*

| ANIMAL NO. | GAIN WEIGHT | GAIN HEIGHT | TOTAL ALFALFA | SILAGE | CORN | BONE MEAL | DIG. PRO. | T. D. N. | CALCIUM | PHOSPHORUS | AV. DAILY CA | AV. DAILY P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *lbs.* | *cms.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *gms.* | *gms.* | *gms.* | *gms.* |
| D–3 | 443 | 36.9 | 1,972 | 3,625 | 1,095 | — | 332.0 | 2,567.2 | 16,664.4 | 3,553.0 | 37.0 | 7.9 |
| D–5 | 442 | 41.9 | 1,948 | 3,352 | 1,097 | — | 324.6 | 2,503.0 | 16,338.7 | 3,431.5 | 36.3 | 7.6 |
| D–7 | 473 | 41.1 | 2,049 | 3,461 | 1,102 | — | 337.3 | 2,577.5 | 17,210.0 | 3,533.5 | 38.2 | 7.9 |
| D–9 | 457 | 36.3 | 2,032 | 3,727 | 1,092 | — | 337.9 | 2,610.2 | 17,267.4 | 3,586.2 | 38.4 | 8.0 |
| D–11 | 456 | 35.1 | 2,049 | 3,483 | 1,098 | — | 340.4 | 2,603.9 | 18,165.9 | 3,656.9 | 40.4 | 8.1 |
| D–13 | 452 | 34.7 | 1,994 | 3,418 | 1,111 | — | 332.1 | 2,552.3 | 17,582.9 | 3,581.6 | 39.1 | 8.0 |
| D–15 | 404 | 33.9 | 1,838 | 3,345 | 1,103 | — | 313.7 | 2,451.1 | 16,701.5 | 3,424.7 | 37.1 | 7.6 |
| Total | 3,027 | 259.9 | 13,882 | 24,411 | 7,698 | — | 2,317.1 | 17,865.2 | 119,930.8 | 24,767.4 | | |
| Average | 432.4 | 37.1 | 1,983 | 3,487 | 1,111 | — | 331.0 | 2,553.0 | 17,133.9 | 3,538.0 | 38.1 | 7.9 |

*Lot II. Basal ration plus bone meal*

| | *lbs.* | *cms.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *gms.* | *gms.* | *gms.* | *gms.* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D–2 | 555 | 39.9 | 2,169 | 4,578 | 1,172 | 55.55 | 368.2 | 2,898.9 | 26,345.5 | 7,166.9 | 58.6 | 15.9 |
| D–4 | 469 | 35.3 | 2,149 | 3,879 | 1,126 | 54.42 | 354.0 | 2,724.5 | 25,505.5 | 6,570.4 | 56.7 | 14.6 |
| D–6 | 504 | 43.2 | 2,106 | 3,715 | 1,115 | 51.55 | 347.0 | 2,664.6 | 24,790.2 | 6,609.8 | 55.1 | 14.7 |
| D–8 | 502 | 34.9 | 2,146 | 4,281 | 1,147 | 56.06 | 359.8 | 2,813.0 | 25,889.7 | 7,016.3 | 57.5 | 15.6 |
| D–10 | 514 | 35.8 | 2,142 | 4,251 | 1,104 | 54.29 | 356.8 | 2,771.3 | 25,336.4 | 6,963.8 | 56.3 | 15.5 |
| D–12 | 476 | 33,7 | 2,126 | 3,794 | 1,107 | 52.51 | 352.8 | 2,690.9 | 25,802.5 | 6,957.8 | 57.3 | 15.5 |
| D–14 | 466 | 36.5 | 2,117 | 3,766 | 1,120 | 52.47 | 351.4 | 2,689.3 | 25,784.1 | 6,901.0 | 57.2 | 15.3 |
| Total | 3,486 | 259.3 | 14,955 | 28,264 | 7,891 | 375.85 | 2,491.0 | 19,252.5 | 179,453.9 | 48,186.0 | | |
| Average | 498 | 37.0 | 2,136 | 4,038 | 1,127 | 53.7 | 355.7 | 2,750.3 | 25,636.3 | 6,883.7 | 57.0 | 15.3 |

TABLE 3

*Summarized data showing average growth in weight and height at withers, food consumption, theoretical phosphorus retention and grams of phosphorus per 100 pounds of live weight from birth to 24 months of age.*[1]

*Lot I. Basal ration low in phosphorus.*

| | WEIGHT AT BEGINNING OF PERIOD | HEIGHT AT BEGINNING OF PERIOD | ALFALFA | SILAGE | CORN | BONE MEAL | DIG. PRO. | T. D. N. | AV. DA. CA | AV. DA. P | THEORETICAL[2] P RETENTION | P PER 100 LBS. LIVE WEIGHT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *lbs.* | *cms.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *gms.* | *gms.* | *gms.* | *gms.* |
| Birth to 3 mo. | 89 | | | | | | | | 9.4 | 6.3 | 2.9 | 3.5 |
| 3–6 mo. | 178 | 84.8 | 256 | 154 | 223 | ..... | 48.9 | 352.4 | 27.7 | 5.7 | 2.8 | 2.2 |
| 6–9 mo. | 262 | 95.6 | 417 | 326 | 225 | ..... | 63.8 | 457.0 | 38.1 | 6.4 | 3.0 | 1.8 |
| 9–12 mo. | 354 | 104.6 | 447 | 644 | 225 | ..... | 70.4 | 528.2 | 39.4 | 8.2 | 3.3 | 1.8 |
| 12–15 mo. | 453 | 110.6 | 434 | 1058 | 219 | ..... | 73.2 | 589.9 | 38.3 | 9.1 | 3.1 | 1.7 |
| 15–18 mo. | 549 | 116.7 | 429 | 1305 | 208 | ..... | 74.8 | 624.9 | 46.9 | 9.9 | 2.5 | 1.6 |
| 18–21 mo. | 625 | 121.9 | 452 | 1587 | 237 | ..... | 79.9 | 669.9 | 44.8 | 9.9 | 3.5 | 1.4 |
| 21–24 mo. | 731 | 124.0 | 450 | 1440 | 273 | ..... | 82.9 | 710.3 | 41.8 | 10.6 | 4.1 | 1.5 |
| 24 mo. | 855 | 127.0 | | | | | | | | | | |
| After calving | 852 | 128.5 | | | | | | | | | | |

*Lot II. Basal ration plus bone meal.*

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Birth to 3 mo. | 91 | | | | | | | | 10.4 | 6.3 | 3.2 | 3.3 |
| 3–6 mo. | 190 | 87.0 | 345 | 188 | 219 | 6.31 | 59.0 | 402.4 | 45.3 | 10.3 | 3.4 | 3.6 |
| 6–9 mo. | 295 | 98.0 | 443 | 413 | 225 | 9.19 | 67.5 | 485.6 | 53.6 | 12.7 | 2.8 | 3.3 |
| 9–12 mo. | 381 | 106.8 | 449 | 805 | 223 | 11.05 | 72.3 | 556.8 | 56.0 | 15.6 | 3.6 | 3.2 |
| 12–15 mo. | 494 | 113.0 | 449 | 1241 | 215 | 13.04 | 76.6 | 627.1 | 60.1 | 18.1 | 3.4 | 3.0 |
| 15–18 mo. | 598 | 118.9 | 450 | 1390 | 245 | 13.96 | 80.4 | 678.4 | 69.8 | 19.7 | 3.0 | 2.9 |
| 18–21 mo. | 688 | 124.0 | 452 | 1425 | 270 | 14.28 | 82.7 | 705.6 | 67.5 | 20.2 | 3.0 | 2.6 |
| 21–24 mo. | 779 | 126.8 | 500 | 1535 | 270 | 16.33 | 89.1 | 750.3 | 70.7 | 22.4 | 4.6 | 2.6 |
| 24 mo. | 858 | 129.9 | | | | | | | | | | |
| After calving | 918 | 130.9 | | | | | | | | | | |

[1] Theoretical P retention and grams of P per 100 lbs. live weight calculated on weight at end of period.
[2] Based on phosphorus in total carcasses of calves as reported by Hogan and Nierman (56).

### *Health*

Other than the difference in depraved appetite and lack of appetite for roughage there was very little difference between the low and high phosphorus animals as far as health and appearance were concerned.

### *Blood Analysis*

The analysis of the blood of both groups of heifers were made for calcium and inorganic phosphorus at 90 days of age and have been continued at two-week intervals since that time. A study has been made of the changes which have been produced on blood calcium and phosphorus by the

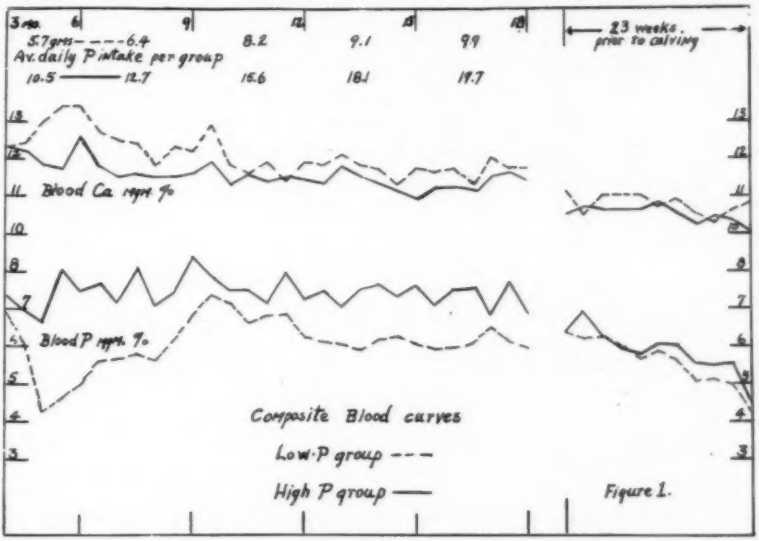


FIG. 1

rations during (a) growth, (b) pregnancy and (c) lactation. This report deals with growth and pregnancy up to the time of first parturition.

### *Time of Taking Blood Samples*

The blood samples were taken under as uniform conditions as possible. The animals were kept in the barn at night and were bled in the morning about three hours after being fed. In this way the effect of exercise was reduced to a minimum; however, some animals struggled severely prior to bleeding. Havard and Reay (51) have pointed out that ordinary movements cause large variations of the inorganic phosphate in human blood. Palmer and co-workers (52) have reported that (a) exercise causes a marked change in the blood phosphate of cattle, (b) feeding causes the in-

organic blood phosphate values to rise within an hour and apparently they do not return to normal until after three hours. This may explain some of the abnormal values which we have recorded.

### *Method of Analysis*

The blood used for analysis was drawn from the jugular vein, without stasis, in a tube containing a small amount of lithium citrate. The blood was immediately centrifuged and the plasma was removed for analysis. For calcium and phosphate determinations, the proteins were precipitated with trichloracetic acid and then centrifuged for ten minutes to complete the precipitation. The supernatant liquid was filtered and the clear filtrate was used for analysis. Calcium was determined from 10 cc. aliquots of filtrate (equivalent to 2 cc. of plasma) by neutralizing to methyl-red as recommended by Shohl (53), by a modification of the Kramer-Tisdall method (47). Inorganic phosphate was determined from five cc. aliquots of filtrate by the Briggs (54) modification of the Bell-Doisy method.

A composite curve for the two groups from three to 18 months of age and for 23 weeks prior to calving is shown in figure 1.

## DISCUSSION

The purpose of this investigation is to determine the phosphorus requirement of dairy cattle when fed alfalfa hay as the principal source of protein along with corn silage and corn. This type of ration furnishes sufficient protein and energy for normal growth and milk production to about 40 pounds of milk per day. However, phosphorus deficiency in Michigan (indicated by depraved appetite) usually occurs when the ration is made up of alfalfa hay, cereal grains with or without silage.

Alfalfa hay is rich in nitrogen which stimulates growth and milk production, but alfalfa hay is low in phosphorus and does not furnish sufficient phosphorus to meet the increased requirement. The phosphorus analyses in table 1 indicate that alfalfa hay grown in many localities is low in phosphorus. When alfalfa hay is supplemented with protein concentrates high in phosphorus such as wheat bran, cottonseed meal, linseed meal, soy bean oil meal or corn gluten meal there is less likelihood of encountering a phosphorus deficiency among dairy cattle. Alfalfa hay containing less than 0.2 per cent phosphorus was used in this investigation. The heifers in lot I suffered from a lack of phosphorus from three months of age to 18 months of age as shown by depraved appetite for bones and wood, also by the lack of appetite for alfalfa hay and corn silage.

The inorganic blood phosphorus of the heifers in lot I (low phosphorus ration) also indicated a phosphorus deficiency. The composite results of the blood pictures of the fourteen animals are shown graphically in figure 1. A decided difference is shown in the blood pictures of the two

groups. The outstanding characteristic of the blood of all the animals on the low phosphorus ration was the immediate drop of the inorganic blood phosphorus from an average value of 6.9 mg. per cent to 4.2 per cent within one month. After the initial drop, the blood phosphorus gradually increased until it had reached an average maximum value of 7.3 mg. per cent by the time the animals were nine months of age. From nine months of age until the animals were 18 months of age there was a tendency for the blood phosphorus to decrease. In comparison with the normal group, the blood phosphorus values did not at any time come up to the normal values during the first 18 months.

As soon as the animals in lot II (normal phosphorus ration) were placed on the experiment, the average blood phosphorus value decreased temporarily from 7.4 to 6.6 mg. per cent and then increased to 8.0 mg. per cent. It should be noted that the increase in blood phosphorus was not due to an increase in phosphorus in the ration but probably due to the change in the ration from milk to steamed bone meal. Such observations have been previously reported (47). Large fluctuations were common during the period up to nine months of age, at which time the average maximum value was 8.3 mg. per cent. From 9 to eighteen months of age there was a tendency for the blood phosphorus curve to decrease.

The blood calcium of cattle has usually been considered fairly constant irrespective of the variations in the intake of calcium. Blatherwick (55), however, has reported a higher value for blood calcium when an animal was on a diet of alfalfa hay than when on a ration of corn silage or grain. He believed this to be due to the greater quantity of calcium in the alfalfa. Our animals in lot I did not consume the quantity of alfalfa hay that the animals in lot II consumed but the blood calcium values did increase during the first three months to an average maximum value of 13.4 mg. per cent. There was a gradual decrease from that time until the animals had become 18 months of age. It is interesting to note that the blood calcium curve remained above that of the normal group in spite of the fact that the average hay consumption of lot I was less than that of lot II. The highest calcium values occurred during the period when the inorganic phosphorus values were the lowest.

Although the heifers in lot I showed evidence of phosphorus deficiency yet they made just as good growth in height at withers from three months to 18 months as those in lot II fed the basal ration supplemented with enough bone meal to bring the phosphorus in the ration up to 0.41 per cent of the dry matter which McCollum (50) considered an optimum level. However, the animals in lot II made an average gain of 66 pounds more per head in body weight than those in lot I on the low phosphorus ration. This difference was probably due to the lack of appetite for roughage exhibited by the low phosphorus animals.

The seven heifers in lot I consumed 2,318 pounds of digestible protein and 17,867 pounds of total digestible nutrients, while those in lot II consumed 2,491 pounds of digestible protein and 19,252 pounds of total digestible nutrients from three months to 18 months of age. The seven heifers in lot I consumed 1,073 pounds of alfalfa hay and 3,853 pounds of corn silage less than was consumed by those in lot II.

Apparently a phosphorus intake of from 5.7 to 9.9 grams daily during the first 18 months was not sufficient for normal physiological functioning under the conditions of this experiment. When alfalfa furnishes the principal source of protein along with silage and corn the calcium-phosphorus ratio is high. In lot I the calcium-phosphorus ratio varied from about 4:1 to 6:1. We were unable to determine the effect of this factor on the amount of phosphorus required since by the addition of bone meal to the ration of the animals in lot II the calcium-phosphorus ratio was usually reduced to between 3:1 and 4:1. The optimum calcium-phosphorus ratio for growing calves has not been determined.

Also, in determining the phosphorus requirement of growing dairy cattle, the vitamin D level of the ration should be considered. Recent experimental results (42) (43) (44) show that calves require vitamin D, and that they can use ultra-violet light from the sun. Consequently, heifers turned into sunshine daily would probably require less calcium and phosphorus in their ration than heifers kept out of sunshine. In this experiment the heifers received about seven hours of sunshine daily when the weather would permit. We also fed five pounds of alfalfa hay cured in the sun which furnished some vitamin D. Steenbock and co-workers (45) have demonstrated that irradiated hay possesses antirachitic properties. Recent results at the Michigan Station (44) indicate that sun-cured timothy hay furnishes sufficient vitamin D to protect calves kept out of sunshine from developing rickets.

Henderson and Weakley (6) reported that heifers fed six pounds of hay per day on a low phosphorus ration and also kept out of sunshine developed badly worn teeth. However, in our experiment the heifers in lot I received less phosphorus per day than reported by Henderson and Weakley, yet their teeth are normal. The differences between our results and those of Henderson and Weakley can probably be explained on the basis of exposure to sunlight, since these investigators also observed worn teeth among their heifers receiving a high phosphorus ration. It is likely that six pounds of hay cured in the sun does not furnish an optimum amount of vitamin D for growing heifers.

Theiler and co-workers (16) secured fair growth in two yearling heifers fed about five grams of phosphorus per day. These investigators stated that their animals were turned into brilliant sunshine daily. No doubt the

amount of sunshine and the amount of sun-cured hay in the ration influence the phosphorus requirement. Had we fed more hay, or hay in place of silage, the phosphorus received by the heifers in lot I might have been sufficient.

Under the conditions of this experiment about 10 grams of phosphorus per day appeared sufficient to maintain the blood phosphorus of the heifers in lot II from three to six months of age. It is unfortunate that we used 0.41 per cent of the dry matter in the rations as phosphorus as our standard for optimum. These animals in all probability received entirely too much phosphorus in their ration as they became older.

Most of the heifers in lot I were normal at about 18 months of age and the blood phosphorus values remained as high as that of the heifers in lot II during pregnancy, although lot II received about twice as much phosphorus. These results suggest that the phosphorus requirement of a growing heifer is not in proportion to body weight. Table 3 shows that the grams of phosphorus in the ration per 100 pounds of live weight were about 2.2 grams from three to six months of age for the heifers in lot I, during which time they showed evidence of the greatest phosphorus deficiency, while at 18 months of age when they were normal only about 1.5 grams of phosphorus were fed per 100 pounds of live weight. This was true even though the animals were pregnant and some phosphorus was required for fetal development.

Theiler (16) was of the opinion that the phosphorus requirement was slightly reduced with age. There is a possibility that the phosphorus requirement for growth depends quite largely on the rate of growth rather than on the basis of body weight. Hogan and Nierman (56) analyzed the bodies of new born calves, at 3, 8, 40, 45, and 48 months of age, for calcium and phosphorus. Some animals were in good condition and some in medium and poor condition. The degree of fatness affected the percentage of calcium and phosphorus in the animal but for the cattle of about the same condition of flesh the per cent of these two elements remained fairly constant for all ages. This indicates that the rate of growth is a good criterion to use in determining the phosphorus requirement for growth. Although the phosphorus required for maintenance is not known, nevertheless it must be very low viewed in the light of Theiler's results when five grams of phosphorus per day not only furnished enough for maintenance but also sufficient for fair growth and reproduction over a period of two years. In table 4, using the data presented by Hogan and Nierman (56), we have estimated the amount of phosphorus required for normal gain in body weights according to Eckles' normals (57). The gain in body weights of our heifers, however, presents almost a straight line since our animals did not grow at a normal rate during the first year of the experiment.

TABLE 4

*Showing phosphorus requirements for normal growth to 18 months of age, based on Eckles' normals and Wilson's requirements for maintenance and growth*

| AGE | NORMAL[1] WEIGHT | P REQUIREMENT FOR MAINTENANCE[2] | AVE. DAILY GAIN WEIGHT | P IN DAILY GAIN | P REQUIREMENT FOR GAIN[3] | TOTAL P REQUIREMENT |
|---|---|---|---|---|---|---|
| *Months* | *lbs.* | *gms.* | *lbs.* | *gms.* | *gms.* | *gms.* |
| Birth | 90 | .64 | | | | |
| 1 | 121 | .86 | 1.03 | 3.04 | 6.08 | 6.94 |
| 2 | 157 | 1.11 | 1.20 | 3.54 | 7.08 | 8.19 |
| 3 | 200 | 1.42 | 1.43 | 3.16 | 6.32 | 7.74 |
| 4 | 249 | 1.77 | 1.63 | 4.80 | 9.60 | 11.37 |
| 5 | 302 | 2.14 | 1.77 | 5.22 | 10.44 | 12.58 |
| 6 | 349 | 2.48 | 1.63 | 4.80 | 9.60 | 12.08 |
| 7 | 389 | 2.76 | 1.33 | 3.92 | 7.84 | 10.60 |
| 8 | 425 | 3.02 | 1.20 | 3.54 | 7.08 | 10.10 |
| 9 | 466 | 3.31 | 1.37 | 4.04 | 8.08 | 11.39 |
| 10 | 501 | 3.57 | 1.17 | 3.45 | 6.90 | 10.44 |
| 11 | 529 | 3.76 | .93 | 2.74 | 5.48 | 9.24 |
| 12 | 558 | 3.96 | .97 | 2.86 | 5.72 | 9.68 |
| 13 | 574 | 4.08 | .53 | 1.56 | 3.12 | 7.20 |
| 14 | 596 | 4.23 | .73 | 1.90 | 3.80 | 8.03 |
| 15 | 612 | 4.35 | .53 | 1.56 | 3.12 | 7.47 |
| 16 | 643 | 4.57 | 1.10 | 3.24 | 6.48 | 11.05 |
| 17 | 660 | 4.69 | .57 | 1.68 | 3.36 | 8.05 |
| 18 | 686 | 4.87 | .87 | 2.57 | 5.14 | 10.01 |

[1] Eckles' Normals (57).
[2] Wilson's Requirements for Maintenance (61).
[3] Twice the phosphorus in gain.

The phosphorus requirement in the ration according to Kellner's idea should be two to three times the retention. Twice the amount of phosphorus required for normal body growth would probably furnish sufficient phosphorus for a young normal growing calf but with increase in size and with decrease in rate of growth this figure would not suffice.

Under Michigan conditions our data indicate that 10 grams of phosphorus per day furnishes sufficient phosphorus to first calving, especially when calves are fed whole milk for two months, five pounds of sun-cured alfalfa hay per day and turned into sunshine daily. No doubt with more vitamin D in the ration the animals could get along with much less phosphorus, but with less vitamin D than was furnished, under the conditions of this experiment, the phosphorus level in the ration should probably be more than ten grams per day to first calving.

The phosphorus levels fed to the heifers in lots I and II produced no effect on the age of the animal at first heat period, days the calf was carried, and the weight and strength of calves at birth. Apparently an intake of 10 to 12 grams of phosphorus per day is about sufficient to support maintenance, normal growth, and gestation from 18 months of age to first calving.

Theiler (16) and Eckles and co-workers (5) have reported that rations low in phosphorus do not support normal reproduction. It is apparent that

the level of phosphorus fed to the heifers in lot I was fairly low in phosphorus but was not sufficiently low to affect reproduction.

During the gestation period the blood curve of the low phosphorus group follows very closely to the curve of the normal group. The blood calcium and inorganic phosphorus values were within the normal limits in both groups. Throughout pregnancy there is a greater demand for both calcium and phosphorus for the growth and development of the fetus. An examination of the composite blood curves indicates that the calcium and the phosphorus requirement were being met during the early months of pregnancy. During the latter months, the curves show that the calcium requirement was apparently adequate but the animals on the low phosphorus ration were evidently receiving barely enough phosphorus for the needs of the cow and fetus.

The curves also show the usual tendency of the blood calcium and inorganic phosphorus to be slightly subnormal during the terminal months of pregnancy (52) (58) (59) (60). The average blood calcium values of the normal and low phosphorus groups immediately following parturition were 10.0 mg. and 10.8 mg. per cent respectively, while the average blood phosphorus values were 4.5 and 4.2 mg. per cent respectively.

## SUMMARY

(1) This experiment was designed to determine the phosphorus requirement of dairy cattle when a ration of alfalfa hay with less than 0.2 per cent phosphorus, corn silage, and corn was fed.

(2) The low phosphorus ration caused an immediate lowering of the inorganic blood phosphorus. These values remained lower than normal during the first eighteen months of age.

(3) The blood calcium values were highest during the time that the phosphorus values were lowest. The calcium values were slightly higher than the normal values during the first 18 months of age but were still within normal range.

(4) Other symptoms of phosphorus deficiency exhibited by the heifers in lot I (low phosphorus ration) were depraved appetite and a reduced appetite for hay and silage.

(5) The ration fed the heifers in lot I which contained from 5.7 to 9.9 grams of phosphorus per day from three to 18 months of age was insufficient when the calcium-phosphorus ratio was from 4:1 to 5:1 and when the sources of vitamin D were five pounds of sun-cured alfalfa hay and sunshine.

(6) Ten and three tenths grams of phosphorus per day appeared to furnish sufficient phosphorus for the heifers in lot II fed the basal ration supplemented with bone meal from three to six months of age.

(7) Ten to 12 grams of phosphorus daily furnished sufficient phosphorus for normal growth, maintenance, and for the development of the fetus from 18 months of age to first calving as indicated by blood phosphorus and the strength and size of calves at birth.

(8) During gestation the blood calcium and phosphorus values were normal for both groups. The blood phosphorus values of both groups decreased markedly immediately prior to parturition.

(9) The phosphorus requirement for growth is not directly proportional to body weight but probably depends on the rate of growth. Consequently, the method used in this experiment of feeding about 0.2 per cent of the dry matter as a low phosphorus ration to the animals in lot I and 0.41 per cent phosphorus as optimum phosphorus level to those in lot II is not a satisfactory method of studying phosphorus requirement for growth. However, under practical farm conditions in which alfalfa hay and the cereal grains furnish protein and energy, calves consume more food as they grow older even though the rate of growth declines.

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# LABORATORY STUDIES ON THE CHEMISTRY OF SOFT CURD MILK

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Much interest in soft curd milk has developed since Hill (1) showed how to differentiate the milks of individual cows with respect to curd character and indicated the potential value of soft curd milk for infant feeding. Hill (2) (3) has since devoted most of his attention to studies on the distribution of curd character in herds, environmental and hereditary factors, and to the clinical aspects of his findings.

Espe (4) (5) has made an excellent experimental study of the digestibility of milk in relation to curd character and has given some attention to the chemistry of soft curd milk.

Our work carried on independently of the above workers consists primarily of analytical and physico-chemical studies on the constitution of soft curd milk.

## PART I. THE QUANTITATIVE COMPOSITION OF SOFT AND HARD CURD MILKS IN RELATION TO CURD CHARACTER

A fairly thorough study was made of the concentration of casein, fat, whey proteins, calcium, phosphorus, citric acid, and lactose in relation to curd character. These constituents will be dealt with both individually and, as far as possible, collectively, in this portion of the paper.

The curd character was determined by means of the Hill test. This test affords a fairly quantitative measure of the compactness of the milk curd upon peptic digestion. It is fully described by Hill (1) in one of his bulletins. Briefly, it consists of adding a standard reagent consisting of 3 parts of an 0.6 per cent pepsin solution and 1 part of calcium chloride solution (378 grams made up to one liter) to 100 cc. of milk brought to body temperature. The milk coagulates rapidly about a set of radial knives which are drawn up through the curd and resistance offered is read off on a spring balance in grams. The readings are most readily reproducible for milks that give a loose curd (soft curd milks) so that one may check on duplicate determinations exactly or within 5 grams. With milks that give a relatively stiff curd the accuracy of reproduction is lower, duplicate readings may vary approximately 10 grams. When the milk is extremely hard curded the variations may be considerably greater.

Received for publication July 7, 1932.

### A. *Concentration of Casein*

Casein determinations were made by the method of Waterman (7) on several soft and hard curd milks. The data obtained are presented in table 1.

TABLE 1

*Concentration of casein and whey proteins in relation to curd character*

| COW NO. | CASEIN | WHEY PROTEINS | RATIO : $\frac{\text{CASEIN}}{\text{WHEY PROTEINS}}$ | CURD TENSION |
|---|---|---|---|---|
| | *per cent* | *per cent* | | *gms.* |
| 32–51 | 1.85 | 0.64 | 2.9 | 28 |
| 10–5 | 2.10 | 0.64 | 3.3 | 28 |
| 20–5 | 2.17 | 0.51 | 4.2 | 30 |
| 4–5 | 2.17 | 0.61 | 3.6 | 58 |
| 32–4 | 2.24 | 0.89 | 2.5 | 22 |
| 19–5 | 2.36 | 0.61 | 3.9 | 32 |
| 13–5 | 2.49 | 0.64 | 3.9 | 30 |
| 26–5 | 2.66 | 0.86 | 3.1 | 58 |
| 28–5 | 2.91 | 0.72 | 4.0 | 40 |
| 38–5 | 3.07 | 0.70 | 4.4 | 75 |
| 45–5 | 3.07 | 1.05 | 2.9 | 110 |
| 32–52 | 3.19 | 0.78 | 4.1 | 120 |
| 14–4 | 3.32 | 0.90 | 3.7 | 85 |
| 37–5 | 3.51 | 0.76 | 4.6 | 140 |
| 31–4 | 3.71 | 0.92 | 4.0 | 163 |
| 36–4 | 4.25 | 0.98 | 4.3 | 190 |

It is evident from table 1 that on the average the concentration of casein in soft curd milk is definitely lower than in hard curd milk. This same conclusion has been independently arrived at by Hill (8) and by Espe (4). However, inspection of table 1 shows that the correlation between curd tension and concentration of casein is by no means ideal.

Espe states (4) that the variation in the percentage of casein accounts for about 81 per cent of the difference in curd tension between hard and soft curd milks. We do not feel it possible to assign so definite a figure. Nevertheless, it may be said that the low percentage of casein is a very important factor in determining the curd character of soft curd milk but that other factors also operate.

### B. *Whey Protein Concentration*

The percentage of whey protein was determined from the nitrogen content of the filtrate obtained after precipitating the casein by the method of Waterman. In table 1 are presented the results of these determinations as well as the ratio of the casein to the whey protein content.

Some indication may be seen of a slightly lower whey protein content in the soft curd milk but it is not as clearly defined as for the casein. There

is some indication also that ratio of casein to whey proteins is generally lower for the soft curd milk.

A high ratio of whey proteins to casein might conceivably be the basis of soft curded milk. In order to test this idea a milk was analyzed for its casein and whey protein content and then to it were added progressive amounts of whey protein powder obtained from the Division of Dairy Industry, Department of Agriculture. The curd tension was determined for each concentration of whey proteins, the concentration of casein remaining constant. The data obtained are presented in table 3. Series A represents a hard curd milk, Series B, a soft curd milk.

TABLE 2

*Effect of increasing concentration of whey proteins upon the curd tension*

| SERIES A | CASEIN | WHEY PROTEINS | RATIO : $\frac{\text{CASEIN}}{\text{WHEY PROTEINS}}$ | CURD TENSION |
|---|---|---|---|---|
| | *per cent* | *per cent* | | *gms.* |
| | 3.39 | 0.76 | 4.5 | 132 |
| | 3.39 | 0.96 | 3.5 | 142 |
| Cow | 3.39 | 1.16 | 2.9 | 135 |
| No. 335 | 3.39 | 1.36 | 2.5 | 140 |
| | 3.39 | 1.55 | 2.1 | 132 |
| | 3.39 | 1.75 | 1.9 | 128 |
| SERIES B | | | | |
| | 2.27 | 0.51 | 4.5 | 60 |
| 8 parts | 2.27 | 0.71 | 3.2 | 58 |
| No. 335 | 2.27 | 0.91 | 2.5 | 60 |
| with 4 parts | 2.27 | 1.11 | 2.1 | 60 |
| water | 2.27 | 1.30 | 1.8 | 57 |
| | 2.27 | 1.50 | 1.5 | 50 |

The data of table 2 indicate that the ratio of casein to whey proteins can play but a small part, if any, in determining the curd character. Moreover, the data would seem to show that the concentration of whey proteins alone plays a negligible part in determining the curd character.

### C. *Concentration of Fat in Relation to Curd Character*

In table 3 are presented the results of several fat determinations on milks of known curd tension.

It will be seen from table 3 that the average percentage of fat in soft curd milk tends to be lower than in hard curd milk but individual variations are large—for example, one soft milk in the table has 5.6 per cent fat and two hard curd milks 3.8 per cent fat.

Hill (2) presented data which show that the removal of the fat from milk causes an increase in curd tension but that the concentration of fat in a milk is not an index to the curd character.

We believe that no simple correlation between fat content and curd character is to be expected since the distribution of the milk fat in relation to the other milk constituents is probably at least as significant as its concentration. That the manner in which the fat is dispersed varies considerably from one individual milk to another seems to be indicated by our work and work done at the New York Agricultural Experiment Station at Geneva.

Experiments which we have made relating homogenization pressure to curd tension clearly show that increasing the dispersion of the fat of a

TABLE 3

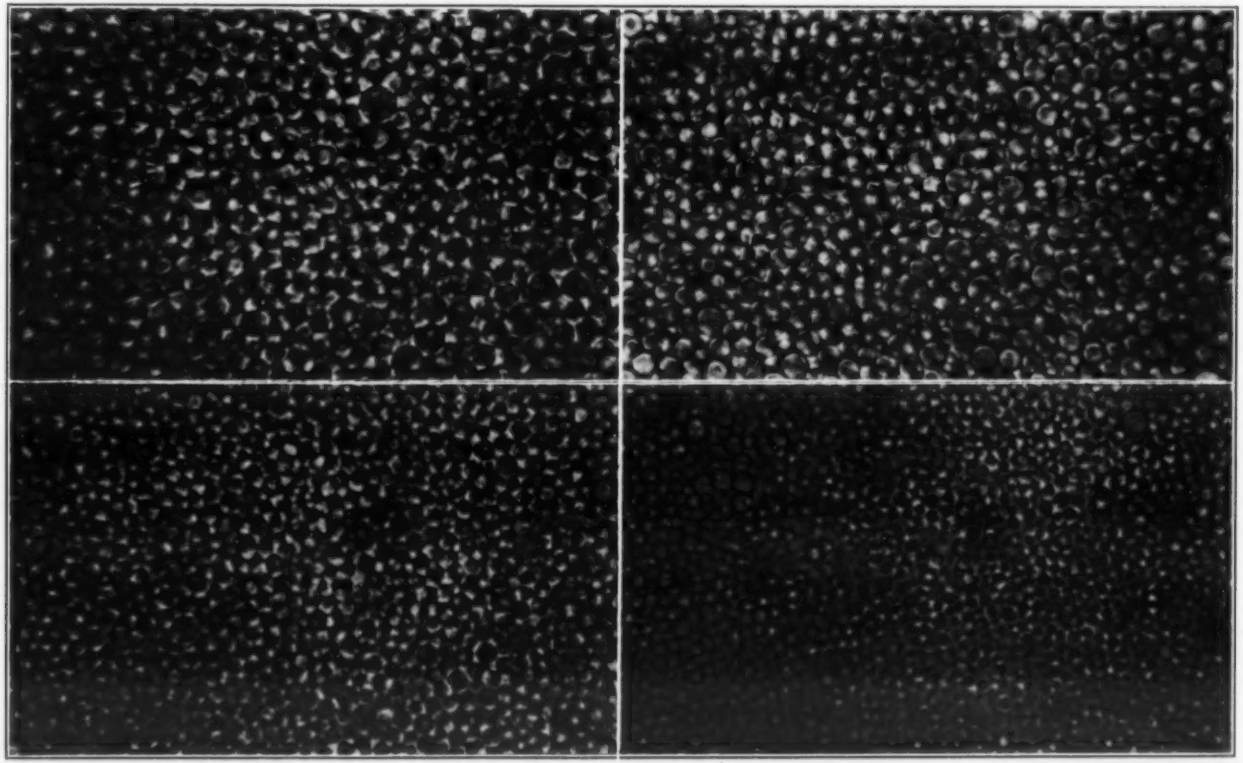
*Concentration of fat in relation to curd character*

| FAT | CURD TENSION | FAT | CURD TENSION |
|---|---|---|---|
| *per cent* | *grams* | *per cent* | *grams* |
| 3.3 | 32 | 6.8 | 110 |
| 3.2 | 39 | 5.9 | 65 |
| 3.4 | 40 | 7.8 | 85 |
| 5.0 | 30 | 5.4 | 75 |
| 4.3 | 30 | 6.8 | 70 |
| 4.0 | 32 | 3.8 | 60 |
| 5.4 | 30 | 7.8 | 55 |
| 3.6 | 25 | 5.8 | 125 |
| 3.6 | 40 | 3.8 | 102 |
| 4.5 | 45 | 4.2 | 102 |
| 4.3 | 38 | | |
| 4.2 | 28 | | |
| 5.6 | 28 | | |
| 4.2 | 30 | | |
| Average 4.2 | | Average 5.8 | |

milk causes a considerable lowering in the curd tension compared with that of the original milk.

Hill's data indicate that the removal of cream may increase the curd tension as much as 20 per cent. The concentration and distribution of the fat unquestionably play an important part in determining the curd character but it is an involved problem to quantitatively evaluate the two effects and we have not attempted its solution at this time.

It seemed a reasonable supposition that the size of the fat globules of the individual milk might bear some relation to the curd character. To test this concept photomicrographs of the fat globules of individual milks were made using a technique similar to that developed by Turner and Haskell (9). No correlation was found between curd character and size of fat globules as may be seen from the photomicrographs.



Size of fat globules in hard and soft curd milks—×744: No. 2, hard curd Guernsey milk (upper left); No. 285, soft curd Guernsey milk (upper right); No. 345, hard curd Holstein milk (lower left); No. 225, hard curd Holstein milk (lower right). Scale, 0.01 mm. = 0.3 inches

### D. *Concentration of Lactose*

In mixed soft curd milk we found the lactose concentration to be 4.8 per cent and in mixed regular certified milk we found 4.7 per cent lactose. Hill (8) found 4.32 per cent in soft curd Holstein milk and 4.68 per cent in hard curd Holstein milk.

In table 4 is shown the effect on the curd tension of adding increasing amounts of pure finely divided lactose.

TABLE 4

*Effect of increasing concentration of lactose on the curd character*

| AMT. LACTOSE ADDED PER 100 CC. RAW CERTIFIED MILK | CURD TENSION |
|---|---|
| *gms.* | *gms.* |
| 0.0 | 58 |
| 1.0 | 55 |
| 2.0 | 55 |
| 3.0 | 60 |
| 4.0 | 45 |
| 5.0 | 50 |
| 6.0 | 50 |

It will be seen from table 4 that the concentration of lactose appears to have little or no effect in influencing the curd tension, unless we assume, what is very improbable, that up to 4 or 5 per cent concentration the lactose does exert an effect and then beyond this concentration no longer does.

### E. *Calcium and Phosphorus in Relation to Curd Character*

Calcium determinations were made according to a method given by Scott (10) and phosphorus determined by a method given in the same book. The data obtained are presented in table 5.

TABLE 5

*Concentration of calcium and phosphorus in relation to curd character*

| DESCRIPTION OF MILK | Ca | $P_2O_5$ |
|---|---|---|
| | *per cent* | *per cent* |
| Mixed soft curd | 0.12 | ..... |
| 32–5 soft curd | 0.09 | 0.23 |
| 7–5 soft curd | 0.11 | ..... |
| 10–5 soft curd | 0.11 | 0.24 |
| Mixed hard curd | 0.15 | ..... |
| 38–5 hard curd | 0.15 | 0.34 |
| 3 hard curd | 0.16 | 0.29 |

It will be noted that both the calcium and phosphorus are present in lower concentration in the soft curd milk but the ratio of calcium to phosphorus is substantially the same for the two types of milk.

### F. *Concentration of Citric Acid in Relation to Curd Character*

According to Sherwood and Hammer (11) the citric acid content of individual milks examined by them varied from 0.07 to 0.33 per cent and the average citric acid content for a given cow's milk seemed to be fairly constant. These facts seemed significant and analyses were made using essentially the same method as that of the above authors.

In table 6 are presented the results of the determinations.

TABLE 6
*Concentration of citric acid in relation to curd character*

| COW NO. | MEAN CURD TENSION | CITRIC ACID |
|---|---|---|
| | *gms.* | *per cent (by volume)* |
| 105 | | 0.23 |
| 195 | 32 | 0.28 |
| 425 | | 0.24 |
| | | mean 0.25 |
| 335 | | 0.28 |
| 375 | 130 | 0.31 |
| 415 | | 0.24 |
| | | mean 0.28 |

The data in table 6 indicate no significant difference in the concentration of citric acid between soft and hard curd.

TABLE 7
*Ash concentration in relation to curd character*

| COW NO. | CURD TENSION | ASH |
|---|---|---|
| | *gms.* | *per cent* |
| 425 | 32 | 0.71 |
| 275 | 39 | 0.68 |
| 415 | 30 | 0.68 |
| | | mean 0.69 |
| 395 | 102 | 0.74 |
| 445 | 102 | 0.69 |
| 184 | 125 | 0.80 |
| | | mean 0.74 |

### G. *Ash Content in Relation to Curd Character*

The amount of ash left after ashing down a sample of milk is an index to the original salt concentration. Table 7 contains some determinations made on hard and soft curd milks.

These data are not comprehensive enough to warrant any definite conclusion. However, according to some data of Hill (8) Holstein soft curd milk contained 0.63 per cent ash and Holstein hard curd milk, 0.74 per cent ash. Espe's data (4) indicate an average of 0.57 per cent ash for soft curd milk and 0.80 per cent for hard curd milk.

### H. *Whey Dilution Experiments*

It was found possible by centrifuging the whole milk in a Sharpless super-centrifuge at 40,000 r.p.m. for 20 minutes to separate the "suspensoid" phase of the milk (the calcium caseinate, colloidal salts, fat) and from the bowl of the centrifuge was obtained a yellowish, transparent whey. From this fluid 93 per cent of the casein was found to be absent.

It was then possible to carry out whey dilution experiments with this liquor by diluting whole milk with it and making curd tension determinations.

In table 8 data are presented to show the effect of diluting a hard curd milk with its own whey and with whey from a soft curd milk.

TABLE 8
*Whey interchange experiments*

| COW NO. | DESCRIPTION OF MILK | CURD TENSION |
|---|---|---|
| | | *gms.* |
| 4–4 | Hard curd whole milk | 122 |
| 4–4 | 70 cc. milk + 30 cc. distilled $H_2O$ | ..... |
| 4–4 | 70 cc. milk + 30 cc. (4–4) whey | 65 |
| 4–4 | 70 cc. milk + 30 cc. (28–4) whey soft curd | 62 |
| 37–5 | Hard curd whole milk | 138 |
| 37–5 | 70 cc. milk + 30 cc. $H_2O$ | 90 |
| 37–5 | 70 cc. milk + 30 cc. (37–5) whey | 75 |
| 37–5 | 70 cc. milk + 30 cc. (24–5) whey soft curd | 88 |

Experiments of this type are naturally not ideal but it was hoped that a rough estimate of the importance of the whey constituents *as a whole* might be obtained in this way. Table 8 indicates that the soft curd whey seems to have the same influence on curd character as the hard curd whey.

To further test this matter, more extensive dilution of the milks was carried out. The data obtained are shown in table 9.

It is difficult to interpret the data of table 9 as the measurements of the Hill test have in general a precision of 5 to 10 grams and the variations fall

TABLE 9
*Supplementary whey interchange experiments*

| DESCRIPTION OF MILK | CURD TENSION | | DESCRIPTION OF MILK |
|---|---|---|---|
| | *gms.* | | |
| 28–4 soft curd whole milk | 69.0 | 102.5 | 4–4 hard curd milk |
| 50 cc. (28–4) milk + 50 cc. $H_2O$ | 22.5 | 27.0 | 50 cc. milk (4–4) + 50 cc. $H_2O$ |
| 50 cc. (28–4) milk + 50 cc. (28–4) whey | 12.5 | 25.0 | 50 cc. milk (4–4) + 50 cc. (4–4) whey |
| 50 cc. (28–4) milk + 50 cc. (4–4) whey | 17.5 | 20.0 | 50 cc. milk (4–4) + 50 cc. (28–4) whey |
| 24–5 soft curd whole milk | 47.5 | 112.0 | 37–5 whole milk (hard curd) |
| 50 cc. (24.5) milk + 50 cc. $H_2O$ | 20.0 | 37.5 | 50 cc. (37–5) milk + 50 cc. $H_2O$ |
| 50 cc. milk (24.5) + 50 cc. 24–5 whey | 10.0 | 30.0 | 50 cc. (37–5) milk + 50 cc. (37–5) whey |
| 50 cc. milk (24–5) + 50 cc. 37–5 whey | 15.0 | 22.5 | 50 cc. (37–5) milk + 50 cc. (24–5) whey |

within this limit. Possibly there is some indication here of a small specific whey influence. However, the dilutions employed in table 9 reduce the concentration of the casein to a point seldom encountered even with a soft curd milk.

## SUMMARY OF PART I

1. The concentration of casein is shown to be lower in the soft curd milk than in hard curd milk. This fact is considered to account, to an important extent, for the lower curd tension of soft curd milk.

2. The soft curd milk contains less calcium and phosphorus than the hard curd milk.

3. The concentration of the individual whey constituents seems to exert little or no influence in determining the curd tension.

4. The whey constituents as a whole likewise seem to exert little if any influence in differentiating a soft curd milk from a hard curd milk.

## PART II. PHYSICO-CHEMICAL MEASUREMENTS ON SOFT AND HARD CURD MILKS

### A. *Freezing Point and Conductivity Measurements*

From measurements of the freezing points and conductivity one might hope to learn something of the "salt balance" of soft and hard curd milks. The characteristic freezing point of a milk is essentially due to the salt ions and lactose. The conductivity depends essentially on the nature and concentration of the salt ions. If any major differences existed between soft curd and hard curd milks in respect to salt balance we might hope to find

indications of it with these measurements. The concentration of lactose we have already seen appears to be quite constant for various milks so its effect may be cancelled out.

In table 10 are presented freezing point data obtained with the Hortvet cryoscope (12). The precision of the measurements is within a few thousandths of a degree. Each value is the result of three or more observations.

TABLE 10
*Freezing point in relation to curd tension*

| CURD TENSION | FREEZING POINT |
|---|---|
| *gms.* | *°C.* |
| 32 | −.545 |
| 38 | −.541 |
| 40 | −.537 |
| 30 | −.535 |
| mean | −.540 |
| 102 | −.535 |
| 102 | −.547 |
| 125 | −.555 |
| mean | −.549 |

There is a mean difference of 0.009° C. in the freezing point of the two types of milk in favor of the hard curd milk which may indicate a somewhat higher salt concentration but a difference as small as this does not indicate a very different distribution of salt ions; moreover, the individual variations in the freezing point do not have a consistent direction.

The conductivity measurements were made with a Leeds and Northrup set-up (1927). The temperature employed was 25° C. and it was maintained with a precision of ± .05° C. The results are presented in table 11.

TABLE 11
*Conductivity in relation to curd character*

| KIND OF MILK | CONDUCTIVITY |
|---|---|
| Hard curd | *mhos.* |
| No. 3 | $42.7 \times 10^{-4}$ |
| No. 38 | $44.8 \times 10^{-4}$ |
| Soft curd | |
| No. 7 | $54.7 \times 10^{-4}$ |
| No. 32 | $51.8 \times 10^{-4}$ |
| No. 19 | $51.6 \times 10^{-4}$ |
| No. 20 | $47.1 \times 10^{-4}$ |

Now and then exceptions have been found but in general the soft curd milks seem to have the greater conductivity.

Table 12 represents an attempt to narrow down the factors responsible for the difference in conductivity. The whey was obtained by centrifuging the whole milk at 40,000 r.p.m. for fifteen minutes. In column A we find a mean difference of 10 units in 48 and in column B, a difference of 6 units in 56, between the soft and hard curd milks. It thus appears that upon removing the suspensoid phase (casein, fat, calcium phosphates) that the conductivity of a soft curd and a hard curd milk tend to approach each other. It will also be noted that the *increase* in conductivity of the whey is greatest for the hard curd milks.

TABLE 12
*Conductivity: whole milk versus corresponding whey*

| KIND OF MILK | A. MILK CONDUCTIVITY | B. WHEY CONDUCTIVITY |
|---|---|---|
| Hard curd | *mhos.* | *mhos.* |
| No. 3 | $42.7 \times 10^{-4}$ | $52.9 \times 10^{-4}$ |
| No. 38 | $44.8 \times 10^{-4}$ | $53.6 \times 10^{-4}$ |
| Mean | 43.8 | 53.2 |
| Soft curd | | |
| No. 7 | $54.7 \times 10^{-4}$ | $59.8 \times 10^{-4}$ |
| No. 10 | $52.7 \times 10^{-4}$ | $58.3 \times 10^{-4}$ |
| Mean | 53.7 | 59.1 |

The conductivity data seem to call for the following interpretation. The greater percentage of poor conducting substances (casein, calcium phosphate and fat) in the hard curd milk causes a lowered conductivity as compared to a soft curd milk. If the influence of the casein, phosphates and fat be cancelled out, there still remains a smaller difference in conductivity which may possibly be explained by the greater dilution of the constituents remaining in the whey of the soft curd milk.

### B. *Viscosity Measurements on Soft and Hard Curd Milks*

It was thought that viscosity measurements might throw some additional light on the central problem. These measurements were carried out with Ostwald pipettes having an emptying time of about 30 seconds for 15 cc. of distilled water. A constant temperature of 25° C. ± .05° C. was maintained by means of a well-insulated bath with an Aminco thermoregulating system. The results are expressed as relative viscosity, distilled water being the reference liquid. In table 13 are the data obtained.

It is evident that soft curd milks have a lower viscosity than the hard curd milks. From the data of table 13, a mean difference of approximately 15 per cent exists in relative viscosity between soft curd and hard curd milks.

Also in table 13 the viscosities of milks are compared with the viscosities of the corresponding wheys obtained from these milks by high speed centri-

TABLE 13
*Relative viscosities of soft and hard curd milks*

| KIND OF MILK | RELATIVE VISCOSITY | RELATIVE VISCOSITY OF WHEY |
|---|---|---|
| Hard curd | | |
| 34–5 | 1.76 | |
| 22–5 | 1.68 | |
| 2–1 | 1.80 | |
| Mixed hard curd | 1.80 | 1.14 |
| Soft curd | | |
| 11–1 | 1.53 | |
| 16–1 | 1.60 | |
| 28–5 | 1.57 | |
| Mixed soft curd | 1.55 | 1.13 |

fuging (40,000 r.p.m., 20 minutes). These data show that the differences in viscosity are associated with the suspensoid phase of the milk (calcium caseinate, calcium phosphates, fat).

These data are then in accord with the analytical data which showed that the concentration of casein, calcium, phosphorus, and often fat, was larger in hard curd milks than in soft; it is to be expected that the greater the percentage of these constituents the greater the viscosity.

### C. *The pH in Relation to Curd Character*

Hydrogen ion concentration has long been known to be an important factor in the phenomena of rennet coagulation (13), even small changes in pH causing large changes in rennet coagulation.

To seek a relation between pH and curd tension, samples of normal milk were rendered alkaline with NaOH and other samples acid with HCl, the pH and corresponding curd tensions were then determined. For the pH

TABLE 14
*Relation of pH to curd tension*

| DESCRIPTION OF MILK | INITIAL pH | pH AFTER CURD TEST | CURD TENSION |
|---|---|---|---|
| 1 cc. N/1 NaOH 250 cc. milk | 7.20 | 5.88 | 12 |
| Untreated milk (control) | 6.75 | 5.80 | 24.5 |
| 2 cc. N/1 HCl 240 cc. milk | 6.35 | 5.54 | 44.5 |
| 4 cc. N/1 HCl 250 cc. milk | 5.95 | 5.36 | 64.5 |

determinations a quinhydrone electrode with a precision of ± .03 pH units was used. The results are given in table 14.

It will immediately be seen that the hydrogen ion concentration seems to have an overwhelming effect on the curd tension. The curd hardness increasing with increasing acidity and diminishing with increasing alkalinity.

Espe (4) found the same sort of effect working independently at Cornell. The next logical step was to determine the pH of various soft and hard curd milks in the hope that the same type of correlation might show itself. In table 15 are shown the results of this work.

TABLE 15
*The pH as found with natural soft and hard curd milks*

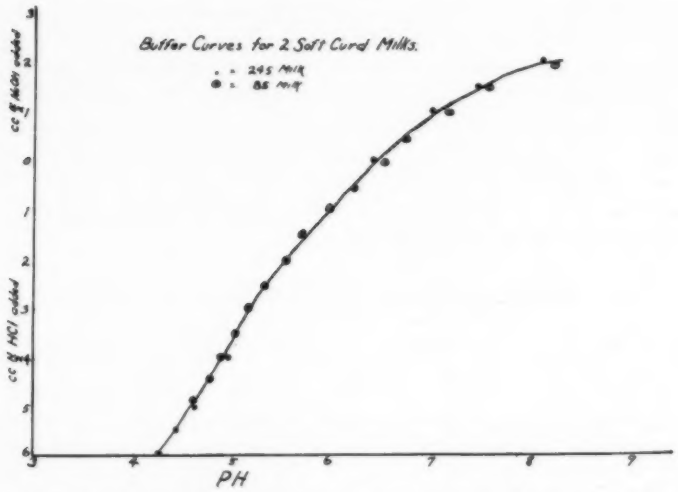
| COW NO. | CURD TENSION | INITIAL PH | PH AFTER CURD TEST |
|---|---|---|---|
| 4–4 | 155 | 6.55 | 5.70 |
| 37–5 | 145 | 6.55 | 5.60 |
| 31–4 | 138 | 6.55 | 5.70 |
| 5–4 | 100 | 6.50 | 5.60 |
| 32–4 | 85 | 6.48 | 5.70 |
| 28–4 | 60 | 6.61 | 5.70 |
| 8–5 | 55 | 6.60 | 5.70 |
| 24–5 | 50 | 6.50 | 5.78 |

The constancy of the pH in untreated milk is such as to eliminate this factor from the problem. Such variations as do exist show no correlation with curd tension. The sharp reduction in pH occurring during the test is owing to the presence of the calcium chloride. Calcium chloride is believed to react with the acid calcium phosphates of the milk liberating hydrochloric acid and tending to form tricalcium phosphate.

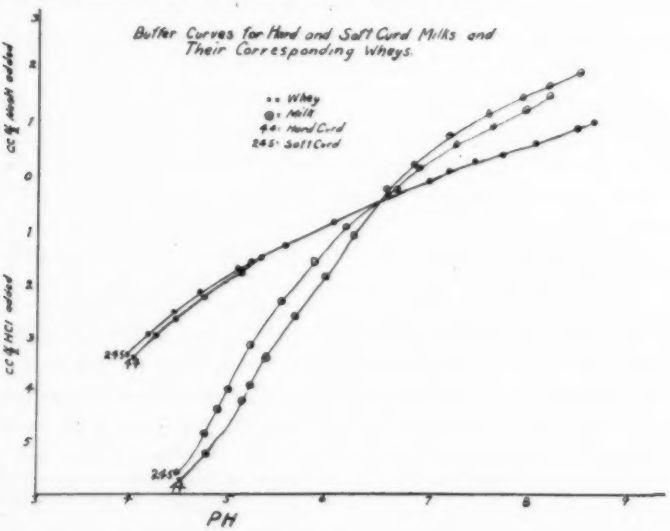
### D. *Buffer Action in Relation to Curd Character*

Milk is a highly buffered system and many properties of milk have been associated with the buffer action of the milk. The chief buffer systems present in milk are the phosphate, citrate, carbonate, and protein (See "Fundamentals of Dairy Science," Chap. VI.) If buffer curves be constructed for a sufficient range of pH the different buffer systems will be brought into play. It was thought that by comparing such curves for hard and soft curd milks something could be learned of the fundamental properties of these milks. The attached curves were obtained by adding definite volumes, from a burette, of N/2 NaOH and N/2 HCl and determining the pH with a quinhydrone electrode the precision of which was ± .03 pH units. Continuous rapid stirring was employed and the instrument was tested with a standard phthalate buffer solution at frequent intervals. Approximately five minutes were allowed for the milk to come to equilibrium before making a reading.

On the same graph were plotted the buffer curves for two soft curd milks (24–5, 8–5) and it was found that the two curves practically coincide




(see Graph I). The buffer curves for a hard curd milk (4–4) and a soft (24–5) were then plotted on one graph (Graph II) and the curves were seen to diverge. The indication was that the hard curd milk was more highly buffered over nearly the whole range, that is, it resisted change in pH more than the soft curd milk. This we would expect from the greater concentration of casein, Ca and P in hard curd milks.



Also on graph II are plotted the buffer curves for the wheys corresponding to 24–5 and 4–4 milks. These wheys were obtained in the customary manner by centrifuging the milks at high speed. It will be seen that the two curves coincide. This indicates clearly that the differences in buffer action were due to the suspensoid phase of the milk (calcium phosphates, fat, casein, etc.).

By plotting data for several hard curd milks, it was found that the buffer curves of the hard curded milks also vary to some extent among themselves. Accordingly, more extensive work will be necessary to confirm the apparently greater buffering action of hard curd milk, relative to soft curd milk.

### SUMMARY OF PART II

1. The freezing point measurements sustain the concept that the salt balance of soft curd milk is not very different from that of hard curd milk.
2. Conductivity, viscosity, and buffer measurements tend to show that the suspensoid phase of the milk is chiefly responsible for the difference in properties between the soft and hard curd milk.
3. The pH seems to bear no relation to the curd tension in natural untreated individual milks.

## PART III. PHYSICO-CHEMICAL EXPERIMENTS WITH SOFT CURD AND HARD CURD CASEINS

### A. *Introduction*

It is conceivable that if there are structural differences in the casein from milk to milk the character of the milk curd would be influenced by such differences, as well as by the differences in concentration of the casein.

In Sutermeister's book (14) the impression is given that the composition of the casein for each species is probably constant, and also that it is constant from species to species. But the analyses on which this conclusion is based undoubtedly were made on mixed milk obtained from large numbers of animals especially in the case of cows, hence this conclusion does not rule out the possibility that the casein of any individual cow may have a composition characteristic for that cow.

In support of this conception is the work of Hugo Meyer (15). He found that in the case of human milk, each mother produces a milk which has a characteristic zone of flocculation at a constant temperature, the pH being varied in definite steps. He then prepared the caseins of each milk and found that these too when suspended in a salt solution had a characteristic flocculation zone which corresponded to the original milk in the sense that casein prepared from milk A always had the same flocculation zone and from milk B a different but characteristic zone.

In table 16 are shown some of his flocculation data.

TABLE 16
*Flocculation zones for individual human milks*

| NAME | FLOCCULATION ZONE |
|---|---|
| Frau Elsa | pH 3.97–5.63 |
| Frau Anna | pH 4.04–5.68 |
| Frau Fanny | pH 3.88–5.29 |
| Frau Lina | pH 3.80–4.72 |

F. Trendtel (16) apparently was able to confirm H. Meyer's work. He found that the isoelectric point of the caseins of individual human milks was found at hydrogen ion concentration varying from $2.19 \times 10^{-5}$ to $7.95 \times 10^{-5}$ and that the isoelectric point was constant for the casein derived from each woman's milk.

We attempted to throw light on this problem by making pure casein preparations from soft curd and hard curd milks and studying their properties.

### B. *Preparation of the Casein from Hard and Soft Curd Milks*

Casein preparations were made from a hard and two soft curd milks employing essentially the method of Van Slyke and Baker (17) except that absolute ether was used as the last wash liquid. With this method the possibility of denaturing the casein is at a minimum. Casein No. 4–5 and casein No. 32–5 were the best preparations obtained. Their analyses are given in table 17.

TABLE 17
*Percentage composition of casein preparations*

| CASEIN | PHOSPHORUS | ASH | PROTEIN | ALBUMIN | LACTOSE | MOISTURE |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | | | *per cent* |
| 32–5 (hard curd) | 0.807 | 0.23 | 90.15 | none | none | 7.73 |
| 4–5 (soft curd) | 0.756 | 0.12 | 91.05 | none | none | 8.05 |

### C. *Tyrosine and Tryptophane Content in Relation to Curd Character*

Casein preparations No. 32 (hard curd), No. 38 (softer curd), No. 4 (softest curd) and "Difco" casein made from mixed cow's milk were analyzed for tyrosine and trytophane by the method of Folin and Ciocalteu (18). (The standards used were made up from Eastman-l-tyrosine.) The data obtained are presented in table 18.

These data seem to show that the tryptophane content is reasonably constant in all the caseins but appreciable variations appear to exist in the tyro-

TABLE 18
*Tyrosine and tryptophane content of individual cow caseins*

| COW NO. | CURD CHARACTER | TYROSINE | TRYPTOPHANE | DRY BASIS | |
|---|---|---|---|---|---|
| | | | | Tyrosine | Tryptophane |
| | | *per cent* | *per cent* | *per cent* | *per cent* |
| 32 | Hard curd | 7.60 | 1.62 | 8.22 | 1.75 |
| 38 | Softer curd | 7.90 | 1.88 | 8.65 | 2.05 |
| 4 | Softest curd | 6.85 | 1.66 | 7.45 | 1.81 |
| * | From mixed milk | 6.40 | 1.58 | 6.98 | 1.73 |

* Digestive Ferments Company.

sine content. However, the variations in tyrosine content do not seem to show a marked trend in relation to curd character.

More extended analyses on the amino acid content of these caseins would seem worthwhile.

### D. *The Isoelectric Points of Soft and Hard Curd Casein*

The isoelectric point of a protein especially in the absence of salts may be expressed in terms of pH. It is the point at which the charge on the protein is zero. The solubility, viscosity, swelling, etc., are at a minimum.

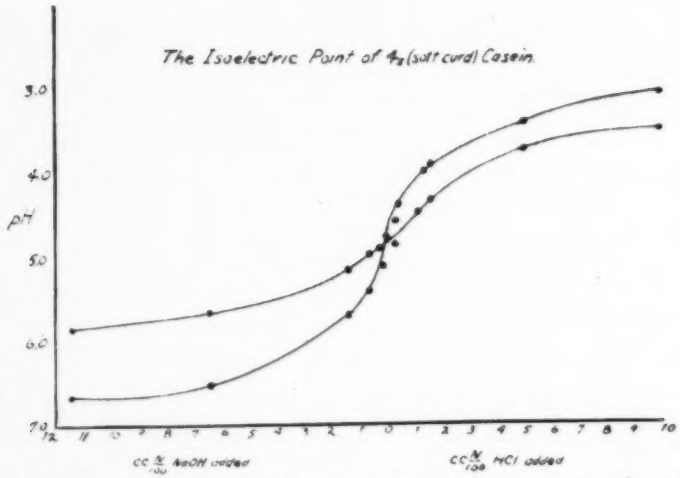
The determination was made in two ways: (1) by simply suspending the casein in $CO_2$ free distilled water and determining the pH with the quinhydrone electrode; (2) by making buffer curves for two concentrations of each casein, 0.50 per cent and 2.0 per cent. When these curves are plotted together they intersect in one and only one point—the isoelectric point. At all other points the difference in concentration of the casein causes a differing buffer action.

In table 19 are shown results obtained by method (1).

TABLE 19
*Isoelectric points of soft and hard curd caseins*

| CASEIN | KIND OF CASEIN | TEMPERATURE | pH |
|---|---|---|---|
| *per cent* | | ° *C.* | |
| 0.5 | No. 32–5 (hard curd) | 28 | 4.74 |
| | No. 4–5 (soft curd) | 28 | 4.74 |
| 1.0 | No. 32–5 (hard curd) | 34.5 | 4.77 |
| | No. 4–5 (soft curd) | 34 | 4.77 |

By method (2) an isoelectric point of 4.85 was obtained for No. 4–5 casein and one of 4.8 for No. 32–5 casein (see Graphs III and IV). The values obtained with $CO_2$ free water are in excellent agreement with those

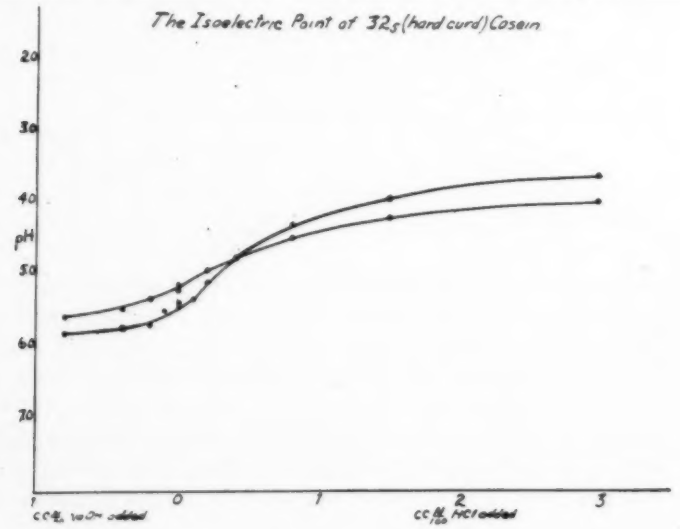



found by Hoffman and Gortner (19) who give 4.77 as the isoelectric pH for casein by this method.

Our data indicate that the soft and hard curd caseins had essentially the same isoelectric point.

### E. *Flocculation Zone of Hard and Soft Curd Casein*

These casein preparations were suspended in a salt solution according to the procedure of S. Marui (20). Using the method of H. Meyer, which con-



sists of adding increasing amounts of N/20 hydrochloric acid, the flocculation zone of the two caseins was then determined. The results are presented in table 20.

TABLE 20

*Flocculation zones of hard and soft curd caseins*

| N/20 HCl (cc.) | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kind of casein | | | | | | | | | | | |
| No. 32–5 (hard) | − | − | − | + | + | ++ | +++ | +++ | ++++ | + | − |
| No. 4–5 (soft) | ± | ± | − | + | + | +++ | +++ | +++ | ++ | + | − |
| pH | | | | | | | | | | | |
| No. 32–5 | | | | | | 4.65 | 4.35 | 4.20 | 3.90 | | |
| No. 4–5 | | | | | | 4.78 | 4.50 | 4.20 | 3.93 | | |

+ indicates flocculation.
− no flocculation.

These flocculation zones are seen to be essentially the same.

### F. *Viscosity of Solutions of Soft and Hard Curd Caseins*

Equal amounts of casein 32–5 (hard) and casein 4–5 (soft) were dissolved in a saturated $Ca(OH)_2$ solution and viscosities determined. In another experiment dry ammonia gas was passed over a layer of each casein preparation in a long glass tube until the compound formed showed a pH of 6.3 to 6.4 in water solution. Then equal amounts of the compound were dissolved in water and viscosity determined. The results are presented in table 21.

TABLE 21

*Viscosity of calcium and ammonium caseinates in relation to curd character. Temperature at 25° C.*

| KIND OF SALT | CASEIN NO. | VISCOSITY |
|---|---|---|
| | | *sec.* |
| Calcium caseinate | 32–5 (hard curd) | 44.0, 44.0 |
| Calcium caseinate | 38–5 (soft curd) | 46.4, 46.4 |
| Ammonium caseinate | 32–5 (hard curd) | 46.3, 46.4 |
| Ammonium caseinate | 38–5 (soft curd) | 46.4, 46.4 |

Table 21 shows no significant differences in viscosity. It was considered, however, that the concentration of casein may have been too small to reveal any differences. The concentration of casein used being about 0.5 per cent.

More concentrated solutions were then prepared using sodium borate which is a good solvent for casein. The results are plotted on Graph V and viscosities are tabulated in table 22.

TABLE 22

*Viscosity of soft and hard curd casein in sodium borate solution. Temperature at 25° C.*

| CONCENTRATION OF CASEIN | VISCOSITY | |
|---|---|---|
| | Casein 4–5 (soft curd) | Casein No. 325 (hard curd) |
| *per cent* | *sec.* | *sec.* |
| 0.0 | 30 | 30 |
| | " | " |
| | " | " |
| 1.0 | 35 | 36 |
| | " | " |
| | " | " |
| 3.0 | 48 | 49 |
| | " | 49 |
| | " | 48 |
| 4.0 | 56 | 60 |
| | " | 60 |
| | " | 61 |
| 5.0 | 74 | 81 |
| | " | " |
| | " | " |

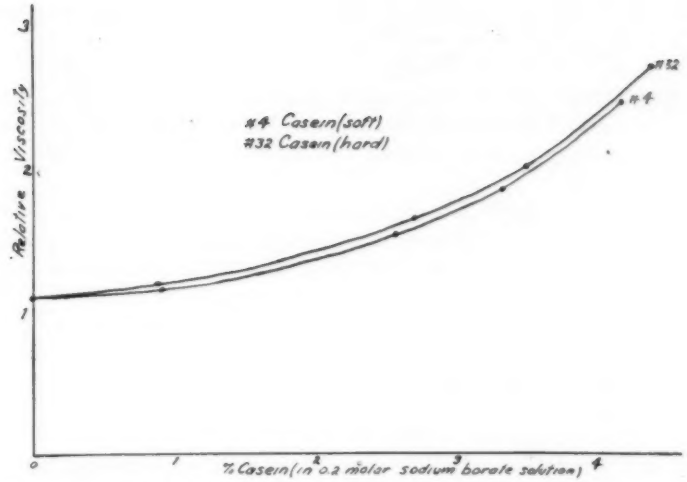
*Note:* The precise concentration of the casein was determined by nitrogen analyses on the solutions. It is these values that are plotted on the graph.

We do not believe that a difference of the magnitude seen on Graph V can be very significant.

The viscosity determinations, the isoelectric point determinations, and the flocculation zones do not serve to differentiate the caseins of the hard and soft curd milk with any sharpness. This however does not prove that the caseins are just alike. It may be that the sensitivity of these methods is not great enough for the purpose. It is conceivable that if a few of the constituent amino acids differed from one casein to another such a difference could not be detected by the determinations described above.

The recent work of Linderstrøm-Lang (21) and Svedberg and Carpenter (22) places the problem in a new light. Svedberg and Carpenter found by means of sedimentation analysis that casein prepared by the method of Hammersten consisted of 3 fractions of differing molecular weight. They, as is customary, doubtless made the casein from mixed milk obtained from several cows.

Linderstrøm-Lang during an extensive research found that casein prepared by the method of Van Slyke and Baker undoubtedly consisted of several fractions. He too doubtless worked with mixed milk from several cows. Linderstrøm-Lang (25) states "For the rest the possibility can not be ex-




cluded that milk from different cows and races of cows can vary in its composition, especially when casein is a mixture of several components, since a change of the proportions of mixing will not be in contrast with the apparently common experience that the chemical units in different kinds of organisms are very closely related to each other."

The direction in which to look for differences in the individual cow caseins thus is pointed out. It may be found to consist in differences in the fractions that go to make up each cow's casein. And these differences may somehow influence the curd character.

## Discussion

By correlating the results of quantitative analysis with measurements of viscosity, conductivity and buffer action on soft and hard curd milks, it seems to be established that the colloidally dispersed phase differentiates a soft curd milk from a hard curd milk. That is, that the fat, casein, and calcium phosphates by reason of their concentration and manner of dispersion seem to control the curd character. (The qualitative make-up of the proteins may also contribute.)

The mechanism by which these constituents determine the difference between soft and hard curd milks may be as follows. The hard curd milk containing more casein should furnish more coagulating centers after peptic action and hence give a more rapid coagulation. In such event the micelles of casein would probably form a closely woven network from which the water would be squeezed out, resulting in a rather dense mass. The soft curd milk on the other hand having less casein would furnish fewer coagu-

lating centers; the micelles would extend farther before interlocking so that a relatively loose structure would result.

The colloidal calcium phosphates severed from the protecting action of the casein by the pepsin, in themselves, may furnish coagulating centers, hence the greater the concentration of calcium phosphates, the more rapid the coagulation, the more compact the micellar structure and the harder curded the milk.

The fat may contribute to the determination of the curd character by interrupting the growth of the micellar threads of protein and so modify the structure. It will be apparent from this viewpoint that the manner in which the fat is dispersed would be as important as its concentration. Another possibility is that the amount of protein adsorbed by the fat varies considerably from individual milk to milk and that this influences the micellar structure which results upon peptic coagulation.

## Final Summary

1. It is established that a high concentration of casein is associated with hard curd milk and a low concentration with soft curd milk.
2. It is clearly indicated that the concentration and manner of dispersion of the fat are important in influencing the curd character.
3. A higher concentration of calcium and phosphorus is found in hard curd milk than in soft.
4. It is demonstrated that the rôle of the whey constituents in determining curd character must be a small one.
5. By correlating the results of quantitative analysis with measurements of viscosity, conductivity and buffer action, it seems to be established that the suspensoid phase differentiates a soft curd milk from a hard curd milk, that is, that the fat, casein, and calcium phosphates by means of their concentration and manner of dispersion seem to control the curd character.
6. Attempts to find decisive differences in composition between caseins from soft and hard curd milks were not successful but the possibility that such differences may exist is not wholly eliminated.

Acknowledgment: The photomicrographs of the fat globules were made by Dr. R. P. Myers in the Research Laboratories of the National Dairy Products Corporation, Baltimore.

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# AN IMPROVED METHOD FOR PREPARING THE SERUM OF BUTTER FOR pH DETERMINATIONS

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Within recent years the correlation of pH to certain qualities of butter has been made by several investigators. The most recent and detailed studies on the method of pH determination of butter are those of Hunziker, Cordes and Nissen[1] and Nissen.[2]

The work of the above authors indicates that determinations of good agreement can be obtained provided the technique is carefully considered. In connection with a study being made at this station on storage butters, it was found that modification of the method recommended by Hunziker *et al.*, which requires the use of one pound of butter, could be made which would introduce a saving in time, and economy in cost, in making the pH determinations.

The reader is referred to the work of the authors mentioned for details of the method presented by them.

The modifications suggested are as follows:

*Modification A*

Measure out approximately 35 grams of a representative and well mixed sample of butter (prepared by making it homogeneous in a water bath at approximately 55° C.) into a Mojonnier flask. Warm in a water bath at 55–60° C. Whirl the flask in the Mojonnier centrifuge for 60 revolutions, taking at least one minute. The serum of the butter will easily and completely separate out in the flask. Pour off the liquid fat as completely as possible and (1) use the retained serum for the pH measurement, or, (2) pour the serum into a clean test tube and chill in cold running water or an ice bath until the small fatty layer remaining is completely solidified. Shake the test tube to insure complete mixture of the aqueous and curdy portion. Insert a pipette through the chilled fatty layer and withdraw the serum. This is then used for the pH determination.

Received for publication July 29, 1932.

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*Modification B*

Measure about 35 grams of a representative and well mixed sample of butter (made homogeneous in a water bath at 55–60° C.) into a test tube having a diameter of about 2.5, and a length of 12, centimeters. Warm the tube and contents a few minutes in the water bath. Whirl in the Mojonnier centrifuge rack, or place the tube, supported and retained by a cork, in a Babcock centrifuge. A small wad of paper or cotton in the bottom of the test bottle cup will eliminate the possibility of breakage of the tube. Whirl for 2 to 3 minutes at a speed of about 200 r.p.m. in an 18 inch centrifuge.

TABLE 1

*The pH* of butter as determined by three methods*

| SAMPLE NO. | A MODIFICATION A USING MOJONNIER FLASK AND CHILLED FAT LAYER | B MODIFICATION B USING LARGE TEST TUBE AND CHILLED FAT LAYER | C METHOD C RECOMMENDED BY HUNZIKER |
|---|---|---|---|
| 18A | 6.407 | 6.415 | 6.430 |
| 18B | 6.648 | 6.582 | 6.599 |
| 19B | 6.349 | 6.357 | 6.299 |
| 20A | 6.474 | 6.466 | 6.474 |
| 20B | 7.072 | 7.072 | 7.072 |
| 22A | 6.357 | 6.364 | 6.349 |
| 23B | 6.467 | 6.466 | 6.482 |
| 211 | 6.349 | ..... | 6.282 |
| 233 | 6.158 | ..... | 6.158 |
| 235 | 6.782 | ..... | 6.773 |
| 243 | 6.131 | ..... | 6.116 |
| 244 | 6.049 | ..... | 6.063 |
| 245 | 6.862 | ..... | 6.865 |
| 246 | 6.782 | ..... | 6.773 |
| 333 | 7.309 | 7.310 | 7.300 |
| 346 | ..... | 5.614 | 5.641 |
| 347 | 5.955 | 6.008 | 5.992 |
| 348 | ..... | 6.192 | 6.150 |
| 350 | ..... | 5.941 | 5.973 |
| 352 | ..... | 5.908 | 5.908 |
| 354 | 7.367 | 7.365 | 7.365 |
| 355 | 5.876 | 5.876 | 5.876 |
| B | 5.641 | 5.657 | 5.641 |
| F | 5.691 | 5.708 | 5.691 |
| X | 6.517 | 6.516 | 6.516 |

* All values determined with the quinhydrone electrode.

(1) Withdraw the serum by means of a pipette inserted through the fat layer, or, (2) chill the contents of the tube in cold running water or an ice bath until the fatty layer has solidified. Then shake or roll the inverted tube between the hands to insure complete mixture of the serum portion and withdraw by plunging a pipette through the solidified layer.

The following table shows the results obtained by three methods on samples with a moderate range of pH values. Column A contains the values obtained by Modification A, the serum being removed through the chilled fat layer. Column B contains the values obtained by Modification B, the

serum being removed after chilling the fat layer. Column C contains the values obtained by the method recommended by Hunziker, Cordes and Nissen, with the exception that glass tubes approximately 4 cm. diameter and 25 cm. length were used instead of the quart jars recommended.

## DISCUSSION

The work of Hunziker *at al.*, indicates that serum was obtainable by centrifugal methods. The above suggestions are, therefore, not original. However, Hunziker and co-workers state that because of difficulty with curd packing against the stop-cock of the separatory flask used, the centrifugal method was abandoned. This objection is met with by use of the Mojonnier flask, or the test tube. It has also been shown that the pH of the aqueous and curdy portion of the serum differ by more than one-tenth pH unit. By solidifying the fatty layer in a test tube, the aqueous and curdy portions of the serum may be thoroughly mixed without incorporation of fat, before removing with a pipette.

It is well known that certain salts, notably calcium, are precipitated from solution upon sufficient increase in temperature. By using the centrifugal method, the quantity of butter required is not only reduced, but the time necessary for preparation of the serum is likewise reduced to a minimum. This may be of importance in making accurate pH determinations of butter made from cream neutralized with lime.

An attempt was made to centrifuge samples containing an immersed electrode. While the method is feasible, difficulties involving fouling of the electrode and calomel bridge were encountered. The modified method is suggested for the assistance it may present in analytical and control work.

## SUMMARY

A method is presented for the preparation of butter serum for pH measurements. The method decreases considerably the amount of butter required. Time and temperature factors are materially reduced.

# CALCIUM AND PHOSPHORUS OF CHEESE MADE UNDER CONTROLLED CONDITIONS

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Cheese is generally recommended as a food of high nutritive value, economical as a source of protein, and rich, not only in protein and fat, but also in calcium and phosphorus. The term cheese, however, includes many products of widely different compositions. The literature contains much data on the composition of cheese made in various ways, the mineral content usually being given as total ash with but few figures for calcium and phosphorus content. The experiments here described were planned to study the calcium and phosphorus content of cheese of several types made under controlled conditions.

Theories concerning the chemical reactions involved in the clotting of milk may be briefly summarized. In the first place, all proteins are recognized as amphoteric substances. Because of this, casein can combine with either acids or bases. In normal milk with a pH on the alkaline side of the iso-electric point of casein, the casein is apparently in combination with calcium as calcium caseinate. According to Bosworth (3) several forms of calcium caseinate may exist. As lactic acid forms in milk, the calcium caseinate changes into calcium lactate and uncombined or iso-electric casein which is insoluble. At the same time the less soluble secondary calcium phosphate of the normal milk changes into more soluble calcium acid phosphate and calcium lactate. In the presence of rennin, or other suitable enzymes, the casein molecule is split, forming molecules of soluble paracasein which unite with calcium to form insoluble calcium paracaseinate. Casein clotted in this way, as calcium paracaseinate on account of the action of rennin, would theoretically contain much more calcium than that clotted as insoluble iso-electric casein due to the change in pH of the solution caused by the formation of lactic acid. The calcium content of cheese made from clotted casein might therefore be expected to vary, depending on the method of manufacture. The work of Wode (16) with rennet cheese demonstrates these points. Rennet was added to some lots only after acid was present, the acidity due to developed lactic acid or to added hydrochloric acid. The percentage of total calcium of the milk retained in the cheese decreased with increasing acidity at the time the rennet was added with consistent losses of calcium in the whey, which has been emphasized by others (15).

Received for Publication, August 2, 1932.
Contribution No. 16, Department of Home Economics
Contribution No. 83, Department of Dairy Husbandry.

Investigations of the phosphorus in casein, recognized as a phosphoprotein, have been thoroughly reviewed (9, 14). The form in which phosphorus is present is still uncertain but it appears highly probable that the combination may be as phosphoric acid. This theory is substantiated by the fact that orthophosphoric acid is readily produced from casein under the action of various mild reagents (10). Linderstrom-Lang is quoted (9) as believing that casein is not an individual substance and has shown that the P/N ratios found in the different fractions are not alike. Hammersten is credited (14) with proving that the phosphorus content of casein is a constant quantity characteristic of the species. The phosphorus content of the casein of cow's milk according to his work is 0.85 per cent. This figure is in close agreement with the more recent work of Berggren (1).

Figures for the percentage of ash constituents of cheese were made available in the table of ash constituents of foods compiled from various sources by H. C. Sherman, appearing in "Chemistry of Food and Nutrition" (13). These figures, including 0.931 per cent of calcium and 0.683 per cent of phosphorus, have been widely used, as in the various editions of the Laboratory Handbook for Dietetics by Rose, given as *Cheese* in the revised edition (11) and as *Cheese, American,* in the third edition (12). Other authors have apparently assumed that these figures hold for various kinds of cheese, in one instance being applied to American pale, Camembert, Cheddar, Full Cream, Neufchatel, and Roquefort alike (4). Blunt and Sumner (2) give interesting data obtained by analyzing commercially prepared cheese. These authors found upon analysis that two rennet cheeses, Swiss and Cheddar, had roughly 14 and 9 times as much calcium as cottage cheese when calculated in terms of percentage and even more when calculated in proportion to the protein. Blunt and Sumner (2) concluded that cottage cheese, unlike hard rennet cheese, must be regarded as a poor source of calcium. However, because some rennet was used in the manufacture of cottage cheese, it is a question whether the difference in composition is due to the rennet alone or to some other variable factor in the manfacture of the product. More recently, Mallon, Johnson, and Darby (6) analyzed American Cheddar cheese in a study on calcium retention and reported figures for calcium content similar to those of Blunt and Sumner (2).

## PROCEDURE

The cheeses used in this experiment were made in the laboratories of the Department of Dairy Husbandry, following as closely as possible the accepted methods of commercial procedure. Records were kept of details of procedure and of results of routine determinations including:

1. Per cent titratable acidity, calculated as lactic acid from titration with N/10 NaOH, phenolphthalein used as an indicator. The acidity was

determined on a sample of whey obtained at the time the curd was drained and cut.

2. Per cent of fat and per cent of total solids by the Mojonnier method.

A sample from each lot of cheese was retained to be further analyzed according to standard methods—the calcium determinations being made according to a modification of McCrudden's method (7), the phosphorus by the method approved by the Association of Official Agricultural Chemists (8), and the nitrogen by the Kjeldahl-Gunning method (8).

Data available were used to prepare tables showing variations in methods of manufacture and also the calories and amounts of fat, protein, calcium and phosphorus in the different products.

Neufchatel cheese, made as indicated in table 1, was allowed to set until coagulation had taken place with a distinct whey layer formed over the curd. The respective lots of cheese were then poured on to draining cloths, allowed to drain as completely as possible, and then weighted down and pressed over night, after which samples were secured. Commercial Neufchatel cheeses sometimes have cream added. Small variations in amount of starter added seemed to have no influence upon the composition of the curd. The lots made were divided into 2 groups, N I, that which had not been so well drained and N II, that which had been drained more completely resulting in a small yield and consequently a greater per cent of total solids and of fat. This cheese, particularly N I, as made in the laboratory had a higher moisture content than commercial Neufchatel, which was due, in part at least, to less pressing in the draining process.

In making cream cheese, each lot of cream was inoculated with a commercial starter, and was held at the setting temperature until the cheese showed an acidity of about 0.7 per cent expressed as lactic acid. As soon as the desired acidity was developed, water equal to 20 per cent of the volume was added to each lot of cheese, and the temperature of the cheese gradually raised to 130° F. in a water bath. Two per cent of salt by weight was added to each lot of cheese during the heating period. The cheese was stirred at frequent intervals until the desired temperature was attained, poured into draining bags and allowed to drain for several hours. Light pressure was applied to the cheese over night and the following morning it was weighed and sampled.

In manufacturing cottage cheese of the rennet type, the cans of coagulated milk were placed in water, the curd was broken, and the temperature of the curd raised slowly to the cooking temperature (120° F.–132° F.). Each lot of chese was held at this temperature until the curd was properly formed. The whey was removed, the curd washed with cold water, spread out on the bottom of a commercial cheddar cheese vat and allowed to drain. At the completion of the draining process the cheese was weighed and samples secured for the analyses reported in this paper. Two of the samples

TABLE 1

*Manufacture of cheeses used*

| KIND | NO. OF LOTS | MATERIAL USED | BUTTERFAT IN MATERIAL | MATERIAL IN ONE LOT | PASTEURIZATION | | STARTER | TEMP. SETTING | RENNET* PER 1000 CC. MILK | ACIDITY | YIELD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TEMP. | TIME | | | | | |
| | | | *per cent* | *pounds* | *° F.* | *min.* | *per cent* | *° F.* | *(cc.)* | *per cent* | *per cent* |
| Neufchatel | 7 | Whole milk | 4 | 8 | 160 | 10 | 0.2–0.4 | 74 | 8–12 | 0.56 | 20.83 |
| Cream | 1 | Sweet cream | 16 | 19.5 | | | | | | | |
| | 6 | | | 30.0 | 160 | 10 | 0.4–0.5 | 70–74 | 0.0 | 0.75 | 36.88 |
| Cottage, rennet | 7 | Skim milk | .05–.10 | 8 | 145 | 30 | 0.7–2.1 | 72 | 1.25 | 0.65 | 23.5 |
| Cottage, acid | 7 | Skim milk | .05–.10 | 8 | 145 | 30 | 0.7–2.1 | 72 | 0.0 | 0.62 | 20.8 |
| Cottage, acid on commercial basis | 6 | Skim milk | .05–.10 | 510–645 | 145 | 30 | 1.5 | 88 | 0.0 | 0.57 | 15.78 |
| Cottage, acid | 1 | Skim milk | | | 175 | 5 | | | | 0.68 | 25.0 |
| | 1 | | | | 160 | 15 | | | | 0.72 | 20.3 |
| | 1 | | | | 145 | 30 | | | | 0.70 | 18.7 |
| | 1 | | .05–.10 | 8 | 145 | 30 | 1.5 | 88 | 0.0 | 0.70 | 20.3 |
| Cheddar | 9 | Whole milk | 3.3 | 800 | 145 | 30 | 2.5 | 86 | 72 | ..... | Approx. 10.0 |

* Liquid rennet obtained from Marshall Dairy Laboratories, Madison, Wisconsin.

of the rennet type of cottage cheese, RC I, did not become so firm during the cooking process as the others, RC II, similar to commercially prepared cheese.

TABLE 2

*Data from analyses of cheese made in the laboratory*

| | NO. OF SAMPLES | CHEESE | CALORIES | PROTEIN (N x 6.38) | FAT | CALCIUM | PHOSPHORUS |
|---|---|---|---|---|---|---|---|
| | | *gms.* | | *gms.* | *gms.* | *gms.* | *gms.* |
| Neufchatel (N I) | 4 | 1.00 | 1.44 | 0.0933 | 0.1183 | 0.00106 | 0.00151 |
| | | 69.4 | 100.0 | 6.48 | 8.21 | 0.074 | 0.105 |
| | | 10.7 | 15.4 | 1.0 | 1.266 | 0.011 | 0.016 |
| Neufchatel (N II) | 3 | 1.00 | 2.21 | 0.1385 | 0.1835 | 0.00102 | 0.00182 |
| | | 45.2 | 100.0 | 6.26 | 8.29 | 0.046 | 0.082 |
| | | 7.2 | 15.9 | 1.0 | 1.321 | 0.007 | 0.013 |
| Cream | 7 | 1.00 | 3.53 | 0.0701 | 0.3613 | 0.00075 | 0.00091 |
| | | 28.3 | 100.0 | 1.98 | 10.22 | 0.021 | 0.026 |
| | | 14.3 | 50.5 | 1.0 | 5.17 | 0.011 | 0.013 |
| Cottage, rennet, (RC I) | 2 | 1.00 | 0.71 | 0.1143 | 0.0102 | 0.00091 | 0.00134 |
| | | 140.8 | 100.0 | 16.09 | 1.44 | 0.128 | 0.189 |
| | | 8.7 | 6.2 | 1.0 | 0.09 | 0.008 | 0.012 |
| Cottage, rennet, (RC II) | 5 | 1.00 | 0.80 | 0.1375 | 0.0102 | 0.00106 | 0.00164 |
| | | 125.0 | 100.0 | 17.19 | 1.28 | 0.133 | 0.205 |
| | | 7.3 | 5.8 | 1.0 | 0.07 | 0.008 | 0.012 |
| Cottage, acid (C I) | 7 | 1.00 | 0.93 | 0.1704 | 0.0103 | 0.00128 | 0.00186 |
| | | 107.5 | 100.0 | 18.32 | 1.11 | 0.138 | 0.200 |
| | | 5.9 | 5.5 | 1.0 | 0.06 | 0.008 | 0.011 |
| Cottage, acid (C II) | 6 | 1.00 | | | | 0.00111 | |
| Cottage, acid (LC 1) | 1 | 1.00 | 0.78 | 0.1373 | 0.0082 | 0.00120 | 0.00161 |
| | | 128.2 | 100.0 | 17.60 | 1.05 | 0.154 | 0.206 |
| | | 7.3 | 5.7 | 1.0 | 0.06 | 0.009 | 0.012 |
| Cottage, acid (LC 2) | 1 | 1.00 | 0.86 | 0.1549 | 0.0089 | 0.00116 | 0.00172 |
| | | 116.3 | 100.0 | 18.01 | 1.04 | 0.135 | 0.200 |
| | | 6.5 | 5.6 | 1.0 | 0.06 | 0.008 | 0.011 |
| Cottage, acid (LC 3) | 1 | 1.00 | 0.73 | 0.1254 | 0.0075 | 0.00110 | 0.00166 |
| | | 137.0 | 100.0 | 17.18 | 1.03 | 0.151 | 0.227 |
| | | 8.0 | 5.8 | 1.0 | 0.06 | 0.009 | 0.013 |
| Cottage, acid (LC 4) | 1 | 1.00 | 0.81 | 0.1451 | 0.0082 | 0.00124 | 0.00147 |
| | | 123.5 | 100.0 | 17.92 | 1.01 | 0.153 | 0.182 |
| | | 6.9 | 5.6 | 1.0 | 0.06 | 0.009 | 0.010 |
| American cheddar | 9 | 1.00 | 4.81 | 0.3417 | 0.3790 | 0.00965 | 0.00365 |
| | | 20.8 | 100.0 | 7.11 | 7.88 | 0.201 | 0.076 |
| | | 2.9 | 13.9 | 1.0 | 1.10 | 0.028 | 0.011 |
| Process | 3 | 1.00 | 4.15 | 0.3778 | 0.2929 | 0.00862 | 0.00356 |
| | | 24.1 | 100.0 | 9.10 | 7.06 | 0.208 | 0.086 |
| | | 2.6 | 10.8 | 1.0 | 0.08 | 0.022 | 0.009 |

For the acid type cottage cheese (CI), the curd was broken up and cooked (100° F.–110° F.) until sufficiently firm. After washing and draining the curd was in the form of relatively fine particles instead of the larger curd particles which are characteristic of the rennet type cottage cheese. For the acid type of cottage cheese made on a commercial basis (C II) the water in the jacket of the vat containing coagulated milk was warmed gradually to a temperature of 140° F. The curd was then cut into cubes approximately three-fourths of an inch square, raised gradually to a temperature 122° F. and held at that temperature until sufficiently firm. The method of manufacture following the completion of the cooking period was the same as for the other groups of cottage cheese. This type of cheese resembles the rennet type cottage cheese very closely. Milk enriched with cream to contain 12 per cent fat was added to this cheese before sampling. Commercial practice varies widely with regard to the amount and richness of milk or cream added before the sale of the product. All other lots of cottage cheese were sampled before the addition of cream so results would be comparable. Data are incomplete for the C II samples which were analyzed at the beginning of the experiment before the nature of this study was fully determined.

Other samples of acid type cottage cheese, group LC, were manufactured as shown in table 2. For LC 4, 0.1 per cent of a stabilizer known as Krabyn was used. Krabyn is a Kraft-Phoenix commercial preparation said to aid in the retention of moisture and in the development of good texture. This sample of Krabyn (5) contained 2.58 per cent of ash, including 0.154 per cent of calcium and 0.034 per cent of phosphorus.

The usual commercial procedure was followed in the manufactured of American Cheddar cheese.

Two groups of processed cheese were studied:

| | NO. SAMPLES | PRODUCT | MADE FROM |
|---|---|---|---|
| Commercial products | 1 | Swiss cheddar processed | |
| | 1 | American cheddar processed | |
| | 1 | Cheese spread | |
| Laboratory products | 1 | American cheddar processed | 1 lot of dry, crumbly, aged American cheddar |
| | 2 | " " " | A mixture of three lots of American cheddar varying widely with respect to age, flavor, body and texture |

To make processed cheese, the cheddar cheese was cut into small pieces and passed through a food chopper. The ground cheese was then placed

in a copper jacketed kettle to which a small quantity of water and emulsifying agent had been previously added. The quantity of water varied from 18.9 to 27.9 per cent of the weight of the cheese. The emulsifying agent, sodium citrate, was varied from 1.8 to 3.0 per cent of the weight of the cheese. The cheese was slowly heated with constant and vigorous stirring to the desired temperature (155° F.–162° F.). The product was sampled and packaged.

## RESULTS

The data from quantitative determinations were used to prepare tables 2 and 3. These have been arranged so that comparisons can be made of

TABLE 3
*Data from analyses of commercial cheese*

| | NO. OF SAMPLES | CHEESE | CALORIES | PROTEIN (N x 6.38) | FAT | CALCIUM | PHOSPHORUS |
|---|---|---|---|---|---|---|---|
| | | *gms.* | | *gms.* | *gms.* | *gms.* | *gms.* |
| Cream | 1 | 1.00 | 3.86 | 0.0880 | 0.3903 | 0.00070 | 0.00096 |
| | | 25.9 | 100.0 | 2.28 | 10.11 | 0.018 | 0.025 |
| | | 11.4 | 44.0 | 1.0 | 4.45 | 0.008 | 0.011 |
| American Process | 1 | 1.00 | 3.67 | 0.2353 | 0.3036 | 0.00695 | 0.00358 |
| | | 27.2 | 100.0 | 6.40 | 8.26 | 0.189 | 0.097 |
| | | 4.2 | 15.4 | 1.0 | 1.28 | 0.029 | 0.015 |
| Swiss Process | 1 | 1.00 | 3.17 | 0.2441 | 0.2430 | 0.00863 | 0.00341 |
| | | 31.5 | 100.0 | 7.69 | 7.65 | 0.272 | 0.107 |
| | | 4.1 | 13.0 | 1.0 | 1.00 | 0.035 | 0.014 |
| Cheese Spread | 1 | 1.00 | 3.03 | 0.2274 | 0.2361 | 0.00649 | 0.00497 |
| | | 33.0 | 100.0 | 7.30 | 7.79 | 0.214 | 0.164 |
| | | 4.4 | 13.3 | 1.0 | 1.04 | 0.029 | 0.022 |

constituents per gram of cheese, per 100 calories of cheese, and per gram of protein in cheese. In calculating calories, the usual physiological fuel values (12) were used:

Protein, 4 calories per gram
Fat, 9 calories per gram
Carbohydrate, 4 calories per gram

Allowance was made for 4 per cent of carbohydrate in cottage cheese.

Whether compared as calories per gram of cheese or as grams of cheese necessary to give 100 calories, the various cheeses show a wide range due largely to the variations in the moisture and the fat contents. For instance, high caloric values are found for American cheddar (4.81 calories per gm.) a concentrated cheese containing 37.9 per cent of fat, and also for cream cheese (3.53 calories per gm.) a moist product containing, however, 36.13 per cent of fat. In contrast, some of the cottage cheeses high in moisture and low in fat have less than 1 calorie per gram. The percentage of fat in the cheese depends upon the material used in manufacture, as:

Cottage cheese (C I) from skim milk, 1.03% fat
Neufchatel cheese (N II) from whole milk, 18.35% fat
Cream cheese, from 16% cream, 36.13% fat

or upon the moisture content as illustrated by two whole milk cheeses:

Neufchatel (N II), moist cheese, 18.35% fat
Cheddar, concentrated cheese, 38.31% fat

Cheeses exhibit a wide range of protein content. For the samples analyzed the figures were approximately:

| | |
|---|---|
| Cheddar and processed cheese made in laboratory | 1/3 protein by weight |
| Commercial cheddar cheese | 1/4 protein by weight |
| Cottage and Neufchatel cheese | 1/6 to 1/10 protein by weight |
| Cream cheese | 1/14 protein by weight |

The calcium and phosphorus in the cheese should be compared on several different bases. In the first place, the percentage or the amount per gram is an important figure, shown as follows:

Soft cheese, about 0.1% Ca, 0.15% P
Cheddar cheese, about 1.0% Ca, 0.3% P

Of the soft cheeses, the cream cheese has the lowest percentages of calcium and phosphorus, which may be explained partly by the high percentage of fat in the product. A comparison on the basis of 100-calorie portions shows the approximate figures:

Cottage cheese, from skim-milk, 0.15 gm. Ca, 0.20 gm. P
Cheddar cheese, from whole milk, 0.20 gm. Ca, 0.074 gm. P
Neufchatel cheese, from whole milk, 0.074 gm. Ca, 0.105 gm. P
Cream cheese, from 16% cream, 0.021 gm. Ca, 0.25 gm. P

To avoid variations due to the fat and moisture content of the products, results have been tabulated as calcium and phosphorus per gram of protein and may be summarized as follows:

Soft cheese, less than 0.009 gm. Ca, more than 0.012 gm. P
Cheddar cheese, 0.028 gm. Ca, 0.010 gm. P

The results obtained from this experiment indicate that phosphorus is proportional to the protein in cheese made from clotted casein regardless of the methods of manufacture, even though they involve different chemical reactions. The Hammersten figure (14) was used, calculating 0.85 per cent of the protein. In cheddar cheese approximately 85 per cent of the phosphorus is accounted for by the protein while in the cottage cheese 75 per cent is calculated as contained in the protein.

Cheddar cheese showed a high calcium content even when calculated on the basis of the protein present. This is consistent with the theories as the rennet, added before the milk became acid, was responsible for the forma-

tion of paracasein which forms an insoluble calcium paracaseinate, resulting in a calcium rich curd. On the other hand, rennet type cottage cheese was not notably higher in calcium than the acid type. This is, no doubt, due to the fact that the rennet, used in only small quantities, has little effect before the milk becomes acid due to the more rapid action of the starter, added at the same time. As lactic acid forms, soluble calcium lactate and neutral casein are produced. The small amount of rennet used had little effect upon the calcium of the curd as it was added only after the other reactions had made considerable progress. Figures for the calcium per gram of protein may be compared:

Acid type cottage cheese, 0.008 to 0.009 gm. Ca
Rennet type cottage cheese, 0.008 gm. Ca
Cheddar cheese, 0.028 gm. Ca

The percentages of calcium and phosphorus of the milk retained in the various types of cheese, as calculated from the yield per 100 pounds of milk, are given:

| | CALCIUM, PER CENT | PHOSPHORUS, PER CENT |
|---|---|---|
| N I | 22.3 | 40.9 |
| N II | 12.8 | 29.4 |
| Cream | 29.2 | 38.6 |
| RC I | 23.5 | 44.0 |
| RC II | 17.6 | 34.7 |
| C I | 21.8 | 40.3 |
| C II | 18.0 | ..... |
| LC 1 | 24.6 | 41.9 |
| LC 2 | 19.3 | 36.4 |
| LC 3 | 16.9 | 32.3 |
| LC 4 | 18.3 | 31.1 |
| American cheddar | 80.4 | 38.1 |

It will be seen in the LC group of cottage cheese in which there was only the variation of the individual pasteurization temperatures, that the percentages of calcium and phosphorus retained decreased according to the decrease of pasteurization temperature which was accompanied by an increase of time. However, only one sample of each type was available for analysis.

In the field of nutrition *shares* are often used as a simple method of presentation of the nutritive values of foods. The share is represented as 1/30 of the average man's daily requirement (12) and specific figures are given for shares in calories, protein, calcium phosphorus and iron. These figures are calculated from the quantities which the average man is said to need: 3,000 calories, 75 grams of protein, 0.68 gram of calcium, 1.32 grams of phosphorus, and 0.015 gram of iron daily. Table 4 gives the values in shares of the various types of cheese analyzed, showing the differences in the protein, calcium and phosphorus supplied by 100-calorie portions.

TABLE 4

*Nutritive values expressed in shares*

1 share in calories = 100 Cal.
1 share in protein = 2.5 gms.
1 share in calcium = .023 gms.
1 share in phosphorus = .044 gms.

| CHEESE | GRAMS | CALORIES | PROTEIN | CALCIUM | PHOSPHORUS |
|---|---|---|---|---|---|
| Laboratory: | | | | | |
| N I | 69.5 | 1 | 2.59 | 3.22 | 2.39 |
| N II | 45.25 | 1 | 2.51 | 2.00 | 1.86 |
| Cream | 28.3 | 1 | 0.79 | 0.91 | 0.59 |
| RC I | 140.8 | 1 | 6.44 | 5.57 | 4.30 |
| RC II | 125.0 | 1 | 6.88 | 5.78 | 4.66 |
| C I | 107.5 | 1 | 7.33 | 6.00 | 4.55 |
| LC 1 | 128.2 | 1 | 7.04 | 6.70 | 4.68 |
| LC 2 | 116.3 | 1 | 7.20 | 5.87 | 4.55 |
| LC 3 | 137.0 | 1 | 6.87 | 6.56 | 5.16 |
| LC 4 | 123.5 | 1 | 7.17 | 5.91 | 4.14 |
| Cheddar | 20.8 | 1 | 2.85 | 8.73 | 1.68 |
| Process | 24.1 | 1 | 3.64 | 9.04 | 1.95 |
| Commercial: | | | | | |
| Swiss Process | 31.5 | 1 | 3.08 | 11.83 | 2.43 |
| American Process | 27.2 | 1 | 2.56 | 8.22 | 2.20 |
| Cheese Spread | 33.0 | 1 | 3.00 | 9.30 | 3.73 |
| Cream | 25.9 | 1 | 0.91 | 0.78 | 0.57 |

## SUMMARY

Fifty-four samples of cheese, 50 being laboratory products made under known conditions, were analyzed for calcium, phosphorus and nitrogen. These included rennet and acid types of cottage cheese, cream, Neufchatel, cheddar and processed cheese. From the compiled data the following points are outstanding:

1. Caloric values vary from 4.81 calories per gram for cheddar cheese and 3.53 calories per gram for cream cheese to less than 1 calorie per gram for various types of cottage cheese.

2. Cheddar and processed cheese made under known conditions are by weight about 1/3 protein, commercial processed cheese 1/4 protein, cottage and Neufchatel 1/6 to 1/10 protein and cream cheese about 1/14 protein.

3. The difference in the percentage of calcium in rennet and acid types of cottage cheese is not significant but cheddar cheese contains approximately 10 times as much calcium as the soft cheeses. About 20 per cent of the calcium of the milk is retained in the soft cheeses while 80 per cent is retained in cheddar cheese.

4. The amount of phosphorus seems to vary according to the amount of

protein in the cheese. About 37 per cent of the phosphorus of the milk is retained in the soft cheeses and 38 per cent in cheddar cheese.

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# THE USE OF VEGETABLE STABILIZERS IN ICE CREAM

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## INTRODUCTION

During the past year numerous inquiries have been received from ice cream manufacturers concerning the merits of certain new ice cream stabilizers of vegetable origin. These stabilizers appeal to some ice cream manufacturers because of their low cost and because there is a demand on the part of some consumers for ice cream which contains no gelatine.

Since only a limited amount of experimental data is available relative to the use of these new stabilizers in ice cream, an investigation of this subject has been conducted during the past year at the Kansas Agricultural Experiment Station. The principal objects of this investigation were: (1) to determine the chemical composition of a representative group of vegetable stabilizers, (2) to observe their effects on certain properties of a standard ice cream mix, and (3) to note their influence on the quality of the finished ice cream.

## METHODS

Five stabilizers of vegetable origin including Krabyn, HyGell, Col-Ace, Sure-Bet, and Kelco Gel were analyzed for moisture, sugar, protein, ash, starch, calcium, phosphorus, and iron, using the methods as outlined by the Association of Official Agricultural Chemists. The gum content of the various stabilizers was obtained by washing with water, to which sufficient alcohol had been added to prevent swelling of the gum. The residue after deducting the ash was considered to be gum. The results of these analyses with the exception of the minerals are expressed in table 1.

TABLE 1
*Analyses of five stabilizers of vegetable origin*

| STABILIZER | MOISTURE | GUM | REDUCING SUGAR | PROTEIN | ASH | STARCH |
|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| A | 10.30 | 28.50 | 54.10 | 2.75 | 2.58 | 0 |
| B | 9.94 | 80.80 | 2.00 | 4.75 | .84 | 0 |
| C | 9.79 | 35.50 | 50.00 | 2.75 | .45 | 0 |
| D | 8.95 | 44.60 | 42.50 | 2.19 | 1.40 | 0 |
| E | 15.36 | 58.80 | 0.00 | 1.31 | 23.65 | 0 |

Received for publication August 6, 1932.
Contribution No. 81 from the Department of Dairy Husbandry, Kansas Agricultural Experiment Station.

The analyses recorded in table 1 would indicate that gums are the active stabilizing agents. The identity of the gums contained in these samples was not determined. The high concentrations of sugar found in samples A, C, and D would indicate that the gum content of these samples had been adjusted so that they could be used in an ice cream mix in approximately the same amounts as gelatine. The sugar also facilitates incorporating the stabilizer into the mix. It will be noted that no starch was found in the samples.

The high ash content of sample E was not accounted for by its calcium, phosphorus, or iron content. Only traces of these three minerals were found in the samples.

Stabilizers designated as D and E in table 1 were not used in ice cream; E was discarded due to the difficulty of incorporating it into the mix and because of the off flavor which it imparted to the ice cream; D was recommended primarily for ices and sherbets. The cost of these two stabilizers would also make their use in ice cream impractical from an economic standpoint.

The quantity of the vegetable stabilizers to use was determined experimentally with small batches frozen in an ice and salt freezer. The amount of stabilizer contained in the respective samples of ice cream which compared most favorably with a portion of the same ice cream stabilized with 0.4 per cent of a 200 Bloom strength gelatine was selected as the desirable amount to use. It was found that 0.35, 0.15, and 0.30 per cent of stabilizers, A, B, and C was required. On the basis of the gum contents of these stabilizers this would be equivalent to 0.099, 0.121, and 0.106 per cent gum, respectively. A knowledge of the gum content of this type of stabilizer is no doubt the most valuable criterion as to the amount to use. When mixed with sugar in dry form and added to the other ingredients at a temperature of 90–100° F., no difficulty was experienced in incorporating the three vegetable stabilizers into the mix.

The vegetable stabilizers were incorporated into a standard 12 per cent butter fat mix and compared with portions of the same mix stabilized with 0.4 per cent of a 200 Bloom strength gelatine and one which contained no stabilizer.

The mixes were heated to 150° F., homogenized at that temperature using 2500 pounds pressure, cooled immediately to approximately 40° F. and aged at that temperature 24 hours prior to freezing.

Each mix was tested for fat, total solids, acidity, and viscosity. The tendency for certain of the mixes to whey off when held at a temperature of 40° F. was observed. Melting resistance studies were made on samples of the finished ice cream.

The fat and total solids determinations were made with a Mojonnier tester. The titratable acidity of the mixes was determined by titrating a 9

gram sample of the mix with N/10 NaOH using phenolphthalein as an indicator. The results are expressed in terms of lactic acid.

Viscosity determinations were made on the fresh mixes and after aging for 20 hours at 40° F. Two viscosity determinations were made on the aged sample, one before stirring and the second after the sample had been stirred with a malted milk mixer for a period of one minute. All viscosity determinations were made with a Mac Michael viscosimeter at 20° C., using a 3 cm. bob and a No. 27 wire.

In studying the stability of the mixes with respect to whey separation, during the aging period, 100 cc. cylinders were filled with the respective mixes immediately after cooling. The cylinders were held at 40° F. and observed at the end of 24, 48, 72, and 96 hours, respectively.

The freezing trials were made after the mixes had aged 24 hours. A 40 quart direct expansion freezer operated with an ammonia temperature of −5° F. was used. Uniform sized batches consisting of 45 pounds each were frozen. A preliminary batch of ice cream was frozen in all cases before freezing any of the experimental batches to insure uniform freezing conditions for all mixes. Overrun determinations were made at minute intervals after the first two minute period had elapsed with a Mojonnier overrun tester. Temperature observations were made throughout the freezing and whipping process by means of a centigrade thermometer graduated to 0.1° C. The time required to obtain 100 per cent overrun was determined with a stop watch.

In determining the melting resistance of the ice cream samples, No. 50 souffle cups, filled at the freezer, were used. Funnels were inserted into 100 cc. graduated cylinders and covered with a ¼ inch mesh wire screen to hold the samples. The samples after hardening were exposed to an average room temperature of 80° F. and the time required for the first drop to fall from the sample was taken as a measure of their melting resistance.

## RESULTS

The results of these determinations and observations as recorded in table 2 represent the average results of four separate trials with each mix used.

It will be observed from the data recorded in table 2 that the mixes were practically identical with respect to their composition and titratable acidities.

The viscosity of the fresh mixes containing the vegetable stabilizers was somewhat greater than that of the other two mixes. After aging for 20 hours at 40° F. the viscosity of all of the mixes was increased, but the greatest increase occurred in the mix stabilized with gelatine. Mixes four and five showed very little change in viscosity as a result of the aging process. It is of interest to note that these same two mixes lost very little of their

TABLE 2

*Effect of different stabilizers on certain physical, chemical and freezing properties of a standard ice cream mix*

*(Average of 4 trials)*

| Mix number | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Stabilizer, kind | Check | Gelatine | Vegetable | Vegetable | Vegetable |
| Stabilizer, designation | ..... | ..... | A | B | C[1] |
| Per cent stabilizer used | 0.0 | 0.40 | 0.35 | 0.15 | 0.30 |
| Fat, per cent | 11.83 | 11.66 | 11.93 | 11.87 | 11.82 |
| Total solids, per cent | 36.62 | 37.33 | 36.95 | 37.08 | 36.96 |
| Titratable acidity, per cent | 0.206 | 0.206 | 0.203 | 0.202 | 0.201 |
| Viscosity, Centipoises | | | | | |
| Fresh mixes | 11.50 | 28.50 | 56.20 | 43.20 | 35.20 |
| Aged mixes before stirring | 12.30 | 76.50 | 81.70 | 48.00 | 38.50 |
| Aged mixes after stirring | 10.50 | 26.70 | 39.60 | 44.00 | 37.20 |
| Whey separation at the end of | | | | | |
| 48 hours, per cent | 0.00 | 0.00 | 0.00 | 1.50 | 12.00 |
| 96 hours, per cent | 0.00 | 0.00 | 0.00 | 8.50 | 25.00 |
| Time to obtain 100 per cent Overrun, minutes | 9.25 | 11.75 | 12.63 | 12.50 | 12.36 |
| Temperature of ice cream when drawn from the freezer, °F. | 24.20 | 24.21 | 24.23 | 24.28 | 24.28 |
| Melting resistance in minutes | 14.40 | 22.20 | 23.40 | 22.40 | 23.80 |

[1] Refer to table 1 for the analyses of these stabilizers.

viscosity as a result of being stirred for one minute with a malted milk mixer. The mix stabilized with gelatine suffered the greatest loss in viscosity as a result of this treatment, being followed by mix number three.

None of the mixes were abnormally viscous nor difficult to handle at any time.

The data presented in table 2 show that an objectionable whey separation occurred in mixes numbered four and five. The amount of separation and the time of its first appearance were not constant for any of the trials. In every case, however, separation had occurred in these two mixes at the end of 48 hours when held at 40° F.

Attempts were made to correct this defect through the use of higher processing temperatures. The amount of separation was decreased but not prevented by pasteurizing the mixes at 170° F. for five minutes. It was found that this whey separation could be prevented in all cases by holding 100 cc. samples of the mix in boiling water for a period of ten minutes immediately after homogenization and cooling. Such a procedure, however, is of no commercial value.

The fact that the use of high temperatures did prevent the objectionable whey separation suggests the possibility that an enzyme may be the active agent responsible for this defect. Additional studies, however, will be required to determine the exact cause of this objectionable whey separation.

The time required to obtain 100 per cent overrun was from 0.6 to 0.9 of a minute longer for the vegetable stabilized mixes than for the gelatine

mix and from 3.11–3.38 minutes longer than for the check mix. The differences in the freezing and whipping properties of the three mixes containing the vegetable stabilizers were insignificant.

The samples of ice cream which were stabilized showed no material differences in their ability to resist melting when exposed to an average room temperature of 80° F. The samples melted down normally in all cases. Laboratory trials in which the amount of the various stabilizers were varied in a standard mix indicated that when excessive amounts of vegetable stabilizers are used the mix had a tendency to become thick and viscous, and when frozen would not melt down normally at room temperatures. This does not appear to be true if the amount of stabilizer used is carefully controlled.

As would be expected, the unstabilized sample of ice cream had the least melting resistance of any of the samples.

The quality of the finished ice cream was determined by scoring three sets of samples taken from each mix at the freezer. One set was held in the hardening room at – 10° F. and examined at the end of 48 hours; a second set was examined after being held at + 8° to + 12° F. in an electrically refrigerated ice cream cabinet for two weeks; the third set was exposed in pint sealright containers to an average temperature of 80° F. for 45 minutes daily over a period of six days and examined. The average body and texture scores on the samples are shown in table 3.

TABLE 3

*Body and texture scores on ice cream samples containing different stabilizers and held under different conditions before examination*

(*Average of 4 trials*)

| MIX NUMBER | STABILIZER USED | BODY AND TEXTURE SCORES | | | AVERAGE BODY AND TEXTURE SCORE |
|---|---|---|---|---|---|
| | | Samples from hardening room | Samples from dealer's cabinet | Heat shocked samples | |
| 1 | Check | 21.50 | 19.00 | 20.00 | 20.16 |
| 2 | Gelatine | 23.00 | 22.50 | 22.25 | 22.58 |
| 3 | Vegetable, A | 23.00 | 22.60 | 23.00 | 22.86 |
| 4 | Vegetable, B | 22.75 | 22.00 | 22.25 | 22.30 |
| 5 | Vegetable, C | 22.87 | 22.75 | 22.50 | 22.70 |

It will be noted from the scores shown in table 3 that the ice cream containing the vegetable stabilizers compared favorably with that stabilized with gelatine and was decidedly superior to that which contained no stabilizer in body and texture.

No off flavors which could be attributed to the stabilizers were detected in any of the samples.

These results would indicate that the stabilizing action of the vegetable stabilizers used in this study was equal to that of the gelatine used.

## SUMMARY

1. The chemical analyses of five ice cream stabilizers of vegetable origin would indicate that gums are the active stabilizing agents in these products.

2. The amount of the various vegetable stabilizers to use in an ice cream mix must be carefully adjusted so that the mix will contain approximately 0.1 per cent gum.

3. The vegetable stabilizers used in this study had no significant effects on the acidity, viscosity, or composition of a standard ice cream mix.

4. The mixes containing the vegetable stabilizers did not freeze and whip as rapidly as the mix stabilized with gelatine.

5. An objectionable whey separation occurred with two of the mixes containing vegetable stabilizers, when they were held at 40° F. for 24 to 48 hours after manufacturing.

6. The melting resistance of the samples of ice cream containing the vegetable stabilizers was essentially the same as for the mix stabilized with gelatine.

7. There was virtually no difference in the quality of the finished ice cream stabilized with the vegetable stabilizers used in this study as compared with that stabilized with gelatine.

## CONCLUSION

The conclusion would seem justified that the principal advantage of substituting vegetable stabilizers for gelatine in commercial ice cream is in their lower cost. This conclusion will only apply to the vegetable stabilizers used in this investigation and under comparable conditions.

# CLARENCE HENRY ECKLES

APRIL 14, 1875—FEBRUARY 13, 1933

Clarence Henry Eckles, foremost American educator and scientist in the field of dairying, died at St. Paul, Minnesota, on February 13, 1933. His passing closed the chapter on a life which has left an indelible imprint on the dairy industry of this country and upon the science of dairy husbandry throughout the world. Few men are endowed with the judgment and vision possessed by this great teacher, counsellor, scholar, and scientist who devoted his life to the dairy industry and the development of its leaders. He was quiet and reserved, but radiated an inspiration which no man could explain. In conference, his friends and colleagues came to depend on his keen perception, his absolute candor, his unwavering honesty, his sympathetic understanding, and a devotion to the highest ideals. He was a prolific writer and contributed widely to scientific and practical knowledge. His passing is truly a great loss, and there is no one who can take his place.

Dr. Eckles was born on a farm near Marshalltown, Iowa. As a young man he took an active part in local social activities and earned the respect of the community. He graduated from the Iowa State College in 1895 with the degree of Bachelor of Science and was at once appointed assistant in dairy husbandry and dairy bacteriology at the same institution. In 1896 he continued his studies in dairy bacteriology at the University of Wisconsin, but returned to Iowa State College where he received the Master of Science degree in 1897. The same university conferred on him the degree of Doctor of Science, honoris causa, in 1916.

It was during the period 1896–1901 that Dr. Eckles was obtaining the major foundation for his broad interests in the field of dairy husbandry which contributed so greatly to his later success in so many branches of this field. During the last years of this period he was in charge of the work in dairy bacteriology at Iowa State College, had charge of the dairy herd of the college and was instructor in dairy manufacturing for one term at the Massachusetts Agricultural College. The first of the almost innumerable contributions from his very fluent pen had already appeared by the time Dr. Eckles' career at Iowa State College came to a close. The bibliography of his contributions discloses eight Experiment Station bulletins and scientific papers, mostly in the field of bacteriology, published during 1896 to 1901, besides several contributions to technological journals.

The longest chapter in Dr. Eckles' career began with his appointment as assistant professor in charge of dairy husbandry at the University of Missouri in 1901, both the position and the department having been created

by the appointment. During the next five years Dr. Eckles devoted his boundless energy to the building of the dairy industry of the state of Missouri and to the establishment of a strong teaching and research department in the Missouri College of Agriculture. During this period he found time to broaden his own scientific training by a year's study abroad, where he came under the influence of Fleischmann at the Georgia Augusta University at Göttingen, Germany, and under von Freudenreich at the dairy experiment station at Liebefeld, near Berne, Switzerland. The principal dairy centers in Denmark, Sweden, England, Holland, and the Island of Jersey were also visited, where he familiarized himself with the best thought and practices of European dairying. By 1906, when Dr. Eckles was advanced to the professorship, he was able to assert to the dairy interests of Missouri that "Missouri is more than a mule and apple state." In this same vein Dr. Eckles stated in 1919 at a banquet held in his honor in Kansas City, "It is true that when I came to Missouri there was very little interest in dairying, but now it has become a respectable thing for a full grown man to milk a cow in this state." Dr. Eckles' leadership as an educator and scientist, and his outstanding ability to translate dairy science into practical terms unquestionably contributed a major part to the developments to which he referred.

The leadership of Dr. Eckles as an educator during this period is exemplified by the fact that by 1910 young men from other states began to come to him for graduate instruction in the field of dairy husbandry. Thus his influence on dairying in other states soon became manifest. By 1919, when Dr. Eckles left Missouri, one-third of the departments of dairy husbandry in other states were headed by his former students.

In the field of dairy science, the period 1906 to 1919 witnessed a great expansion of Dr. Eckles' interests and contributions. Although dairy bacteriology continued to hold his interest for a time, investigations by him, his staff, and graduate students, pertaining to the effect of food on the chemical composition of milk and milk products, and to the nutrition and physiological development of dairy cattle, soon became his major interest. A file of Dr. Eckles' publications during the period 1901 to 1919 shows 58 scientific contributions as Experiment Station bulletins and journal articles of which he was author or collaborator, and nearly 100 contributions by him to practical farm papers.

The final chapter in Dr. Eckles' career began in 1919 when he accepted the position of Chief of the Division of Dairy Husbandry at the University of Minnesota. After going to Minnesota, his work took on a new momentum, and his leadership in the field of dairying expanded still further. Although the dairy industry in Minnesota was already well organized when Dr. Eckles came to the state, he witnessed a development in dairying so

extensive that the leading agricultural industry passed from the hands of the wheat growers to those of the butter producers. The direct and indirect support of the university group, which he headed, was an important factor in this development.

As an educator, Dr. Eckles continued to attract an increasing number of graduate students, including several men from abroad. The first Doctor of Philosophy degrees in Dairy Husbandry were granted to Dr. Eckles' students during this period when eight received this degree. Six others, who are candidates for this degree, had not completed their work at the time of his death.

Dr. Eckles' active scientific interests in all branches of the dairy industry multiplied and expanded still further so that the scientific contributions in which he collaborated with his staff and colleagues doubled during the period of 1919 to 1933, and had reached a total of 110 at the time of his death. Almost every phase of dairying, including dairy cattle nutrition, dairy production, milk secretion, and dairy bacteriology, are touched upon in the contributions of this period. There were twelve publications alone dealing with various phases of the problem of phosphorus deficiency disease in cattle, which had been one of his major fields of investigation during the past ten years. Even during this time Dr. Eckles maintained his contact with the practical problems of the producers of milk and milk products through many contributions to farm magazines and especially as a member of the editorial staff of several such publications, first as dairy editor of *Farm, Stock and Home,* of Minneapolis, then as corresponding editor of *Farm and Fireside,* New York City, and finally as dairy editor of *The Farmer,* St. Paul.

Dr. Eckles was the author of the text book on "Dairy Cattle and Milk Production," which first appeared in 1912, and which was revised in 1923. It is recognized throughout the world as an outstanding contribution to the knowledge of the care and management of dairy cattle. He was also co-author of "Dairy Farming" with G. F. Warren in 1916, and of "Milk and Milk Products" with W. B. Combs and H. Macy in 1929. In late years, Dr. Eckles had given considerable attention to the assembling of new material which he hoped to use in the revision of these books and for proposed treatises on silage and on the history of dairy cattle. He had looked forward to the time when his administrative duties might be lightened so that he could devote more of his energies to such writing. It is a tragedy that this opportunity never came, as dairy literature would have been greatly enriched by his contributions.

Dr. Eckles was one of the founders of the American Dairy Science Association, which he served as president in 1921. His interest in the development of this association has had much to do with its growth and usefulness.

His influence was strongly felt in the establishment of the JOURNAL OF DAIRY SCIENCE, of which he was an associate editor from 1922 until his death. He was a member of Alpha Zeta, Gamma Sigma Delta, Phi Kappa Phi, and Sigma Xi; the Society of Experimental Biology and Medicine; Fellow of the Iowa Academy of Science; and Fellow of the American Association for the Advancement of Science. He was elected Corresponding Member of the Czechoslovakian Academy of Agriculture in 1931.

Although Dr. Eckles was recognized as a scientific worker, an author, a judge of men, and a wise counsellor, his greatest contribution was in the training of young men to follow in his footsteps. Few men have had the privilege of guiding so many students in the attainment of advanced degrees, and to no other teacher in the field of dairying have so many students come for graduate instruction. A total of 142 students have had at least one year of post-collegiate work under his guidance. Of these, 89 obtained the degree of Master of Arts or Master of Science and eight the degree of Doctor of Philosophy. The respect and esteem of his pupils manifested itself in the unique organization, the Eckles Club, organized in 1916, the present membership of which is 140. His spirit was transmitted to the graduate students who seated themselves about the table with the "Chief" at the head, especially in his class in Dairy Research. His modesty and charm naturally drew men to him and he inculcated in his students a love of research, of honest labor, and a zeal for truth which have gone with these men to all parts of the world and wherever dairying is taught or followed.

Never could one more appropriately say "He was one of God's noblemen."

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# METHODS OF ANALYZING DAIRY PRODUCTS

## FOREWORD

In 1927, the Committee on Bacteriological Methods of the American Dairy Science Association began a series of reports dealing with the various laboratory procedures suitable for the bacteriological analysis of dairy products. This committee has functioned through the agency of sub-committees, composed of those who are especially interested in certain phases of the dairy industry.

The Sub-committee on Bacteriological Methods for Ice Cream published the first of these reports in 1927.[1] This was followed by a report on Microbiological and Chemical Methods of Analyzing Butter in 1930[1] and by one on Bacteriological Methods of Analysis of Dry Milk and Related Powders in 1932.[1]

The revisions of the Ice Cream and Butter Reports as well as the Dry Milk and Related Powders Report are also issued as separates and these may be purchased at cost from the chairman of the general committee, Dr. R. S. Breed, Geneva, New York.

The objective of these committees has been to make available in condensed form various methods for the analysis of dairy products. The views expressed in these reports are those of Committees appointed by the American Dairy Science Association. As such they are printed for the criticism of other members of the Association and other interested persons.

## The Bacteriological Analysis of Ice Cream

### METHODS OF SAMPLING

#### *Sample Containers*

Screw capped vials with metal caps, preferably lined with rubber gaskets, are very satisfactory for milk and similar liquids, especially when samples are to be shipped. Wide-mouthed, ground glass stoppered bottles of about 120 cc. capacity are convenient for collecting viscous samples of the semi-frozen ice cream as it leaves the freezer.

#### *Collection of Samples*

*Liquids.* Cream, milk, skimmilk or ice cream mix should be sampled by means of a sterile glass or metal tube. Such a tube may be of any convenient length and diameter. Metal tubes for use in 40 quart cans are ordinarily ¼ inch inside diameter and 22–24 inches long. Longer tubes may be convenient in some cases. One to four tubes may be completely wrapped

[1] See references on pages 287, 299 and 306.

in paper or enclosed in a metal case before sterilizing. If the liquid to be sampled is in cans of uniform diameter a composite sample may be obtained by agitating thoroughly, using a sterile stirrer or one already in one of the cans; a sterile sampling tube is then slowly inserted to the bottom of the can; a representative sector of the contents is removed and transferred to a sterile sample vial or bottle. If the liquid to be sampled is in a vat, the contents should be agitated thoroughly and then sampled by means of the sterile sampling tube from at least six places.

A sample taken from the spout of the homogenizer or freezer may be obtained by quickly passing the bottle under the opening at periodic intervals during the discharge, so as to get a representative sample of the material as delivered from the machine.

*Solids.* Sugar and gelatin should be thoroughly mixed with a sterile spatula and a portion placed in a sterile sample bottle. Skimmilk powder should be sampled in a similar way, detailed directions being given in the Report of the Sub-committee on Condensed, Evaporated and Dry Milk (5).

Frozen ice cream should be sampled with a sterile trier after removing the ice cream from the surface to a depth of about one inch from the point to be sampled. The core should be cut into short pieces with a sterile spoon or spatula and placed in a sterile container.

### *Care of the Samples*

All samples should be placed in water containing cracked ice and taken to the laboratory for analysis with the least possible delay. Insulated shipping boxes fitted with chilled "brine pads" have been found very satisfactory for shipping frozen samples.

### *Measuring the Sample*

The sample of ice cream may be either measured or weighed. Since it is frequently difficult to expel all the air, one 10 cc. sample may contain more or less ice cream than another. Variations in the viscosity of the mix and the cleanliness of the pipette may result in some of the mix adhering to the glass. Although the use of a weighed sample offsets these difficulties, it has the disadvantage of being more tedious and time consuming. In a statistical analysis of parallel determinations made on the gravimetric and volumetric bases, Fay (9) found that the errors inherent to the plate count overshadowed the variations introduced by the method of sampling. The preference for the gravimetric or volumetric method will depend on the nature of the analytical work. For ordinary control work, where a routine check is being made on the quality of the ingredients and the final product, the volumetric method is sufficiently accurate. On the other hand, for research work, where accuracy and comparability of results are essential, the use of the gravimetric method may be preferable. If consecutive samples

are being taken at the various stages in the process of ice cream manufacture, all determinations should be made on the same basis, either volumetric or gravimetric.

If the sample to be analyzed is frozen ice cream, it should be melted and the air expelled by heating in a water bath at 45° C. (113° F.) for 15 minutes. A higher temperature is likely to injure some of the organisms, whereas a lower temperature may necessitate an exposure which permits appreciable growth. Occasional agitation of the sample during the melting process greatly facilitates the expulsion of the air. When the volumetric method is used for ice cream, extreme care should be taken to insure complete expulsion of the air, and to rinse the pipette several times in the dilution water until reasonably free from ice cream.

*Volumetric method. Liquids.* The larger the volume of sample used in making the first dilution the less will be the percentage error in measuring the sample. This is especially true with liquids of high viscosity such as heavy cream and melted ice cream mix. The amounts most commonly used are: 1 cc. of sample in 99 cc. of sterile water, 5 cc. in 95 cc., or 10 cc. in 90 cc., giving dilutions of 1:100, 1:20, and 1:10 respectively. The volume of the sample plus the volume of the sterile dilution water should equal 100 cc. The dilution, therefore, should be expressed as in "1:10," "1:100," etc., and the results in terms of "plate count per cubic centimeter—50,000, etc." Not more than two significant left-hand digits should be used in reporting counts.

*Gravimetric method. Liquids.* If a 10-gram sample of cream, melted ice cream, condensed milk, etc., is weighed directly into a dilution blank, it does not matter how much adheres to the pipette or how much air remains in the melted ice cream.

A dilution blank containing 90 cc. of sterile water is counterpoised on a torsion balance. (It is convenient to have a beaker of water on each pan of the balance to facilitate counterpoising.) The instrument should be sufficiently sensitive so that one drop of water will disturb the balance. After counterpoising to within one drop of the true balance, add a 10-gram weight to the pan opposite the dilution blank; remove the cap and place it on the scale pan, taking care that it does not touch a non-sterile surface. Under aseptic conditions, slowly introduce the sample until the 10-gram weight is accurately balanced. Test the accuracy of the weighing by adding one drop of the sample in the beaker on the opposite pan; if one drop throws the balance pointer to one side, the weight may be considered sufficiently accurate for use in the analysis. The method described by Parker (13) is advantageous in weighing a sample directly into the dilution bottle. The dilution bottle is fitted with a rubber stopper, which, in turn, is fitted with a glass rod. The rod is bent in such a fashion that it permits the suspension of the stopper during the weighing process.

Another method of preparing a gravimetric sample has been described by Smith, Newman, and Nielsen (15). Briefly, this method consists of accurately weighing a small sample of melted ice cream in a sterile vessel of such size and shape that the vessel, together with the weighed sample, can be dropped into the dilution blank. The bacterial count per gram is then calculated on a basis of the weight of the sample employed. The following quotation is taken from that part of the original description which deals with the method of weighing the sample:

1. Sample to be kept well packed in cracked ice until examined.
2. In each test-tube is placed a metal butter-boat; the tubes then are plugged with cotton and sterilized in the hot-air sterilizer for one hour at 175° C.
3. When cooled, a test-tube containing a butter-boat is placed on one pan of a sensitive balance and accurately weighed to the nearest milligram (0.001 gm.). Record weight.
4. The ice cream sample is taken from the ice and liquefied in warm water—temperature of water not to exceed 40° C. and for a period not longer than five minutes.
5. When the ice cream has become liquefied, rapidly shake the sample 25 times, "each shake being an up and down excursion of about one foot (entire shaking not to take longer than about seven seconds)."
6. Remove cotton plug from test tube, holding the tube in a slanting position so that the sterile butter-boat slowly slides down toward the opening against the plug. Allow about ¼ to ½ inch of the boat to project out beyond the opening of the tube. Replace the tube on the balance pan, with the cotton plug alongside of it.
7. Measure 1 ml. of the liquid ice cream sample with a sterile 1 ml. bacteriological pipette and carefully expel it into the exposed end of the butter-boat.
8. Quickly obtain the weight of the ice cream, weighing to the nearest milligram. Record the difference between the two weights which is the weight of the ice cream.
9. With one hand remove the foil cap from a dilution bottle containing 99 ml. of sterile water, and with the other hand carefully remove the test-tube from the balance pan; then, using care not to spill any of the sample, allow the boat containing the ice cream to slide end first into the dilution bottle. This bottle should be marked with an identification number before proceeding with the next sample.
10. When the samples have been weighed and transferred into their dilution bottles in the manner described above, they should be plated in accordance with the method described for milk in the latest edition of the "Standard Methods of Milk Analysis of the American Public Health Association."

*Gravimetric method. Solids.* The results of gravimetric analyses are reported in terms of "plate count, 50,000 per gram, etc." Analyses of gelatin, sugar, and skimmilk powder are usually made on a gravimetric basis by weighing 10 grams of the dry sample directly into a bottle containing 90 cc. of sterile water; or, into the empty sterile dilution bottle, the

required amount of sterile water being added just prior to dissolving the material for plating. (If the latter procedure is followed, it is necessary to use dilution bottles which may be sterilized with dry heat.) Appropriate dilutions are then made and plated in the usual manner. In the dilution of gelatin it is necessary to warm the blanks to insure uniform distribution of the sample.

Fruits, such as strawberries in heavy syrup, may be weighed into sterile wide-mouthed glass-stoppered dilution bottles or on sterilized paper, counterpoised on a balance. If the fruit is whole, it may even be necessary to grind it in a sterile mortar. If 10 grams are weighed into a sterile bottle either from the original container or from the mortar, 90 cc. of sterile dilution water should be added and the whole shaken vigorously.

## AGAR PLATE METHOD

### *Preparation of Agar Plates*

*Media.* For ice cream analysis, several media have been found satisfactory and others not mentioned here, no doubt, could be used with equal satisfaction. Most public health laboratories, however, use the standard agar as described in detail in the latest edition of Standard Methods of Milk Analysis (1). Briefly, this medium is prepared as follows: Add 0.3 per cent of Bacto-meat extract (or other brands giving comparable results), 0.5 per cent Bacto-peptone (or other brands giving comparable results), and 1.5 per cent market agar to the desired number of liters of distilled water; dissolve by heating in an autoclave for from 40 to 90 minutes after the pressure has reached 15 pounds, the length of time depending on the quantity of medium being prepared. Remove the medium and determine its reaction. The reaction will ordinarily be approximately pH 6.6. Do not adjust the reaction if it falls between pH 6.4 and 7.0. After testing the reaction and adjusting, if necessary, the medium should be filtered through cotton or through paper pulp in Buchner funnels. Place 10 cc. portions of medium in test-tubes or 55 cc. portions in flasks and sterilize at 15 pounds pressure (120° C.) for 20 minutes.

Milk powder agar, as described by Ayers and Mudge (2), has been found advantageous in the determination of different types of bacteria in ice cream.

Carbohydrate media such as dextrose agar, saccharose agar, etc., may be prepared by adding one per cent of the desired carbohydrate to the plain agar previously described. The addition of sugars to the plain medium increases the number and size of the colonies appearing on the plates.

When plain agar is used in plating ice cream it is not infrequent to find a wide disparity in the counts based on different dilutions from the same series of dilution blanks. As illustrative of this point Fay (9) cited a spe-

cific case in which 20,000 colonies were estimated to be on the 1:10 dilution plate, 3000 on the 1:100 dilution plate but none of the 300 colonies expected on the 1:1000 dilution plate could be found. Replating of such samples on carbohydrate agar resulted in the appearance of the expected number of colonies on the plates from the higher dilutions. Fabricius and Hammer (8) recommended the use of one per cent saccharose agar in the plating of ice cream as a means of avoiding these aberrant results in plates made from various dilutions.

If it is desired to check the results of analysis with those obtained in another laboratory, it is essential that the media employed be of the same composition.

*Incubation.* The Standard Methods of Milk Analysis recommends incubation of plates for 48 hours at 37° C. Although a higher count usually will be obtained by incubating 48 hours at room temperature followed by 48 hours at 37° C., this procedure necessitates a larger supply of petri dishes and delays the results. The extra expense may be justified by the special demands of research work, but for routine control work, incubation for 48 hours at 37° C. is recommended. If results are to be compared with those from another laboratory, the incubation time and temperature should be uniform.

*Counting the colonies.* A convenient method is to invert the plate and mark each colony with ink or a wax pencil as it is counted, at the same time keeping a record of the count with a tally meter. This method greatly increases the accuracy of counting by preventing confusion due to interruptions or to counting the same colony twice. All plates in a series should be observed at as nearly the same time as possible.

All counting of colonies should be done with the aid of a magnifying glass. A reading glass four inches in diameter, magnifying at least 1½ times is recommended as satisfactory for use. Low power binocular magnifiers are also advantageous. It is recommended that constant (preferably artificial) illumination be employed while counting the colonies.

### COUNTING BACTERIA IN ICE CREAM BY MEANS OF THE MICROSCOPE

The examination of ice cream under the microscope supplies certain valuable data which can be obtained in no other way. The direct microscopic examination of ice cream may be used to best advantage with the unpasteurized product and with the raw materials used in the mix. It is also advantageous in the detection of the use of moldy fruits in the pasteurized product.

#### *Preparation of Ice Cream Film*

*Volumetric method.* While preparations may be made in the usual way with the 0.01 cc. pipette, Fay (9) has described a modification of the

Breed method which is especially adapted for use in the microscopic examination of cream, ice cream, and condensed milk. One-tenth cc. of the sample is placed on a chemically clean glass slide of standard size (25 × 76 mm.). Two to four drops of sterile or boiled water are added, and the mixture spread evenly over the entire area of the slide. The smear is allowed to dry in the air, fixed, and stained as subsequently outlined in this report.

When the diameter of the microscopic field is adjusted to 0.155 mm., the average number of bacteria per field is multiplied by 1,000,000 to give the count per cc. If the product contains relatively few organisms it is more convenient to count the total number of organisms in a strip the width of the microscopic field across the short dimension of the slide. In this case the total number of cells is multiplied by the factor 5000 to give the count per cc. (A factor of exactly 5000 requires a field with a diameter of 0.152 mm. However, the slightly larger field employed (0.155 mm.) partly offsets the lack of clear delineation at the periphery of the field.)

*Gravimetric method.* Fabian (6) proposed the following adaptation of the Breed method to the analysis of ice cream.

A chemically clean glass slide is carefully counterpoised on an analytical balance and 0.01 gram of the ice cream weighed upon each end of the slide as quickly as possible in order to reduce the error due to evaporation. A platinum loop has been found satisfactory for placing the ice cream on the slide. The 0.01 gram of ice cream is then spread uniformly over an area of one sq. cm., using a clean needle. After the film has been spread, the slide should be placed on a warm surface for drying. The drying should not be so rapid as to cause the film to peel from the slide. However, the drying should be completed quickly (within ten minutes) or growth may take place. The preparation is then ready for staining by one of the methods subsequently described in this report.

If the diameter of the microscopic field is adjusted to 0.205 mm., each field will cover an area of 1/3028 sq. cm. (1/3000 sq. cm. is the approximate figure and the one generally used). When this adjustment is made, about 1/300,000 part of a gram of the dried ice cream is visible in one microscopic field. The average number of bacteria found per field, therefore, should be multiplied by 300,000 to give the estimated number of bacteria per gram. Usually 10 to 20 fields should be examined for each sample of ice cream.

### *Staining Methods*

The staining processes described apply to the smears prepared from a gravimetric or volumetric sample.

*Three-solution technique.* After the smear has been air-dried it is dipped in xylol or other suitable fat solvent for five minutes. (Longer exposure is necessary for ice cream than for milk, due to the larger amount

of fat present.) The xylol is drained off and the slide placed on a warm surface to facilitate evaporation. The slide is then immersed in 70 to 95 per cent grain or denatured alcohol for about 20 minutes. Again place the slide on a warm surface to evaporate the alcohol. Stain for 30 seconds in an alcoholic or carbolated methylene blue solution. The alcoholic solution of the stain is prepared by adding 30 cc. of a saturated alcoholic solution of methylene blue to 100 cc. of distilled water. (It is advisable to use only the methylene blue certified by the Commission on Standardization of Biological Stains.)

The smear is then partially decolorized by submerging in 70 to 95 per cent alcohol until the background is light blue and the cells are dark blue. The length of time required for satisfactory decolorization varies with different smears but is usually only a few seconds. The slide should then be dipped in water to stop the decolorizing action of the alcohol, dried, and examined with a standardized microscope.

*One-solution technique.* The Newman-Lampert method described by Newman (12) and Breed (4) is convenient in that the fixing, staining and decolorizing are reduced to one operation. The formula for the fixing and staining solution is as follows:

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|---|---|
| Methylene blue, certified | 1.00–1.12 gms. |
| Ethyl alcohol, 95 per cent | 54 cc. |
| Tetrachlorethane, technical | 40 cc. |
| Acetic acid, glacial | 6 cc. |

Add the alcohol to the tetrachlorethane and heat to a temperature not to exceed 70° C. Add this solution to the powdered methylene blue. Shake until thoroughly dissolved, then allow to cool to room temperature. Add glacial acetic acid very slowly. Filter and keep tightly stoppered.

Immerse the air-dried smears in the staining solution and remove immediately. Allow to drain for 30 seconds; dry; wash with water, and examine with a standardized microscope. (It is important that the slide be dried before washing with water.)

*Differential staining methods.* Proca (14) and later Beattie (3) developed staining methods for differentiation of living and dead cells. Although these methods have not been found satisfactory for reliable differentiation of the living cells in pasteurized dairy products, unpublished experimental data give promise that this very desirable procedure may be improved still further.

## INTERPRETATIVE VALUE OF BACTERIAL NUMBERS

The total number of bacteria in such dairy products as milk, cream, and ice cream has been used for many years as an index to sanitary quality. The use of this index is based on the assumption that the conditions which favor the admission and growth of bacteria are not in keeping with the

commonly accepted ideas of the manner in which dairy products should be handled. The factors responsible for large numbers of bacteria in ice cream are: poor quality of ingredients, inefficient pasteurization, insanitary equipment, improper aging conditions, and careless methods of the employees. This is to say, ice cream with a high bacterial content has been neglected in one or more of the above points. It is possible, however, that ice cream with a low plate count may have been made from poor quality ingredients, and the fact masked by pasteurizing at a high temperature or for a long time.

A serious difficulty with the index value of bacterial numbers is the unavoidably high percentage of error in any method of estimation yet devised. This fact should always be borne in mind in using the results as an index to insanitary conditions.

To the plant operator, high bacterial counts should indicate that there is something wrong with the methods or the quality of the ingredients. If detailed inspection of the plant does not reveal the cause, it is necessary to take routine samples throughout the manufacturing process to locate the trouble.

High bacterial counts should not be construed by the inspector to mean that the plant is insanitary or that the product is necessarily unsafe, but rather that there is something faulty about the plant methods which can be revealed only by more detailed inspection. On the other hand, ice cream having a low bacterial count should not be regarded as above reproach from a sanitary point of view, due to the possibility of masking improper cleaning methods and the use of poor quality of ingredients by employing high temperature or long time pasteurization. It is always advisable to combine inspection with laboratory findings before conclusions are drawn from the results.

Excessive numbers of bacteria in ice cream are significant largely through the faulty methods or materials which their presence reveals, and not because excessive numbers of bacteria indicate the presence of disease germs. At best, the bacterial count can be used only as an aid to the inspector and not as a substitute for inspection.

### QUALITATIVE METHODS

*Anaerobic spore test.* Weinzirl and Harris (16) have applied the anaerobic spore test to ice cream as an index to manurial pollution. The procedure as adapted to the analysis of ice cream is as follows: One cc. of ice cream is placed in each of five sterile tubes containing about two cc. of sterile vaseline. Approximately 10 cc. of sterile water are then added to each tube. The tubes are heated to 80° C. for ten minutes to destroy the vegetative cells, drive out the oxygen, and to bring the vaseline to the surface, thus affording an anaerobic seal. All tubes are then incubated for 96

hours at 37° C. A positive reaction is indicated by a gassy fermentation in three or more of the five tubes.

The interpretative value of this test is based upon two assumptions, (1) that the chief sources of anaerobic spore-forming bacteria in dairy products are manure and dirt contamination, and (2) that the conditions under which dairy products are ordinarily handled are not conducive to the growth of these organisms, so the number found in the sample approximates the number which gained entrance from these sources. Both of these assumptions have been questioned.

*Colon test.* Several methods have been devised for determining the number of organisms of the colon group in dairy products.

One of these methods consists of plating suitable dilutions on the eosin-methylene blue agar described by Levine (11). Typical colonies are counted and may be transferred for further identification according to the Standard Methods of Water Analysis outlined by the American Public Health Association (1925 and 1933).

Weinzirl and Harris (16) inoculated lactose fermentation tubes with serial dilutions (1:10 to 1:1,000,000) of the sample to be tested. After enrichment, transfers were made to Endo agar plates from all tubes showing ten per cent or more of gas. Typical colonies were transferred to other media for subsequent identification according to the American Public Health Association Methods.

Kessler and Swenarton (10) proposed the use of gentian violet lactose peptone bile for the detection of colon organisms in milk. This medium consists of one per cent each of lactose, peptone, and bile. The reaction is adjusted to pH 7.8 before the addition of gentian violet (1–25,000). The medium is sterilized in fermentation tubes for subsequent use in the serial dilution method.

In the manuscript of the proposed 6th edition of the Standard Methods of Milk Analysis of the American Public Health Association which was provisionally accepted at the Washington 1932 meeting of the Laboratory Section of that Association for publication at the end of 1933, dehydrated brilliant green, lactose bile 2 per cent (Bacto) is recommended for use in milk control work. Undoubtedly this will also come into general use in ice cream work as well.

There is considerable criticism of the colon test as a criterion of the sanitary quality of ice cream and other dairy products. The presence of these organisms in dairy products does not have the same significance as does their presence in water. Dairy products are produced under conditions which preclude the likelihood of complete absence of bacteria of the colon group. The cow, the stable, the milker, utensils and other environmental factors are all sources of contamination with colon organisms.

The problem is further complicated by the prevalence of organisms of the colon group but usually of non-fecal origin. Members of the genus *Aerobacter*, commonly found in the soil and feeds, are distinguishable from the members of the genus *Escherichia* only by special tests. Milk is an excellent medium for both *Escherichia* and *Aerobacter* types, so that once they gain entrance, they may greatly increase in numbers unless properly controlled.

It has been shown by Fabian and Coulter (7) that members of the *Escherichia-Aerobacter* group may be quite resistant to the temperatures ordinarily used in pasteurizing ice cream. There is also considerable variation in the resistance of different strains to the same pasteurizing temperature. These facts should be considered in using the colon test as an index of the efficiency of pasteurization.

Insufficient work has been done with the colon test to justify the establishment of standards for the colon content of ice cream. Until further investigations establish the significance of the presence of colon organisms in ice cream more clearly it is doubtful whether the numerical determination of these types should be employed as a routine procedure.

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# THE MICROBIOLOGICAL ANALYSIS OF BUTTER

## METHOD OF SAMPLING

*Butter in the Churn.* After the butter has been worked and is ready to be removed from the churn, samples for microbiological analysis should be taken by means of a sterile trier, taking three samples of about 1 ounce each, one sample from the center of the churn, the other two from the respective ends.[1] The butter should then be placed in sterile sample jars (screw or glass top).

*Tubs or Packages.* At least a total of 1 ounce of butter should be removed from two different parts of the tub or package with a sterile trier, the plugs of butter to be not less than 2 inches in length and to include the surface portion. The butter should be transferred from the trier to the sterile sample jar with the aid of a sterile spatula or spoon.

*Print Butter.* Because of difficulty in obtaining the same amount of exposed surface of butter on one, one-half, and quarter pound prints, print butter should be sampled by means of a trier. By using a small trier and plugging the end of the print, a plug of 3 to 4 inches may be obtained. Such a plug should weigh at least half an ounce. The butter should be transferred from the trier to a sterile sample jar with the aid of a sterile spatula or spoon.

## CARE OF SAMPLES

All samples, immediately after sampling, should be placed in cracked ice or placed in a refrigerator where the temperature does not exceed 4° C., and plated as soon as possible.

Where samples are procured from a grading station remote from the laboratory, the sample should be stored at −10° C., or lower until shipped.

For shipping, the samples should be cooled, placed in a container, and packed carefully so as to prevent breakage and retain a low temperature. Upon receipt of the samples at the laboratory they should be plated as soon as possible and a record of the number of days elapsed between sampling and plating should be made.

## PREPARATION OF SAMPLES FOR PLATING

Place the sample jar containing the butter to be tested in a water bath between 40 and 45° C., and shake until the butter is of a thin creamy con-

[1] Under commercial conditions where large numbers of samples must be taken daily, it may be impractical to employ a sterile trier for each sample. If a polished trier is wiped thoroughly after each sample with tissue paper until the surface is highly polished and then plunged into the butter to be sampled at least twice before the sample is taken, satisfactory results can be obtained with a single trier.

sistency. The time required for the sample to reach a creamy consistency should not exceed 15 minutes. Plating must follow immediately.

### TREATMENT OF EQUIPMENT

Sterilization of glassware and equipment should correspond to Standard Methods of Milk Analysis. (1) "Glassware, except that containing rubber, cork or other material that will char at the temperature used, shall be sterilized for not less than one hour at a temperature of not less than 170° C. except when it is known by means of recording thermostats that the oven temperatures are uniform under which exceptional conditions 160° C. may be used for not less than 1½ hours." Dilution bottles, media, etc., shall be sterilized in the autoclave at 15 pounds (120° C.) for at least 20 minutes after the pressure reaches 15 pounds.

Metal triers, spatulas, and spoons should be wrapped in paper (imported Kraft wrapping paper usually withstands sterilization temperatures without charring), or enclosed in metal or glass containers and sterilized in the same manner as glassware.

Wooden tongue depressors serve as satisfactorily as spatulas. They may be wrapped in paper or enclosed in a metal or glass container and sterilized by autoclaving, or by hot air.

Equipment such as pipettes, dilution bottles, petri dishes, and water used for dilution blanks should correspond to the specifications of Standard Methods of Milk Analysis (1).

### PREPARATION OF DILUTIONS

With a warm 1 cc. pipette, the melted butter should be drawn up into the pipette, care being taken to have as little butter as possible on the outside of the pipette. To remove as much butter as possible from the pipette, blow hard through the pipette into the dilution blank which has been warmed to a temperature of 40 to 45° C. Rinse the pipette by sucking the dilution water into the pipette.

### DETERMINATION OF YEASTS AND MOLDS

*Media:*

1. Potato Dextrose Agar.
2. Bacto Dehydrated Malt Agar.
3. Bacto Dehydrated Wort Agar,
   or, any medium yielding comparable results.

*Preparation of Potato Dextrose Agar.* Boil for 1 hour in 1,000 cc. of distilled water 200 grams potato which has been previously peeled and sliced. Strain through double thickness of cheesecloth and restore to original volume. Add

2.0 per cent of commercial dextrose.
1.5 per cent of agar agar.

Dissolve in autoclave and sterilize.

The medium should be sterilized in definite quantities, preferably 100 cc.

*Acidulating the Medium.* Adjust the pH of the hot sterile agar to 3.5 (2) with sterile tartaric acid at a definite concentration (dissolve 10 grams of U. S. P. tartaric acid in 100 cc. of water); then cool the solution before pouring. Never heat the agar after the addition of the acid, as heating at a pH of 3.5 destroys the jellying properties of the agar.

The amount of tartaric acid to be used varies according to the buffering qualities of the medium.

Potentiometric methods should be used to determine the amount of acid necessary to bring the medium to pH 3.5, but when not available colorimetric methods may be substituted, using the indicator, brom phenol blue, at which a yellowish green color is obtained at approximately pH 3.5.

*Dilutions.* Dilutions suggested for determining the yeast and mold content of butter are 1, 0.1, and 0.01 of a cc. Higher dilutions may be made if necessary.

*Plating.* About 10 cc. of acidulated melted agar cooled to a temperature of 40 to 45° C. should be poured into each petri dish after the introduction of the sample and thoroughly mixed with the sample by a rotary motion.

The plating should be completed before the solidification of the butter in the plate, in order that an even distribution of the sample may be obtained.

After the medium has thoroughly hardened, invert the petri dishes and place in incubator.

*Incubation.* The plates should be incubated at 21 or 25° C. for 5 days (4).

After two or three days the plates should be examined for mold growth and, if the molds are developing in large numbers, a count should be made at this time and a recount made on the fifth day.

*Counting.* Count the number of mold and yeast colonies without the aid of a counting lens.

*Reporting Results.* The number of yeast and mold colonies per cubic centimeter should be reported together as a total count. The medium used should also be recorded and the temperature used for incubation.

Johns, C. K. (7), has developed a modification of the Frost Little Plate Method for determining the yeast and mold count of butter. This technic was given in the first report (8) of the Committee on the Microbiological Analysis of Butter.

TOTAL BACTERIAL COUNT

*Medium.* The following medium is suggested for the determination of the bacterial count in butter:

1. Beef Infusion Agar plus 1 per cent lactose pH 6.6 – 6.8 made with fresh beef or Bacto beef.

Recent unpublished results by White (11) show that of the media studied highest counts were obtained on beef infusion agar prepared from fresh beef or Bacto beef.

A. For the preparation of beef infusion agar the following directions are given on the basis of one liter.

Solution A:

1. Infuse one pound of lean chopped beef in 500 cc. of distilled water and hold overnight at from 0 to 10° C.
2. Secure the infusion by squeezing through a cloth or pressing (the following rapid procedure is suggested: pour off the free juice and heat the meat and remaining juice until hemoglobin is destroyed, filter or strain, add the juice to first portion); if less than 500 cc. is obtained, make up to this volume with distilled water.
3. Warm to 50° C.

Solution B:

1. Dissolve 15 grams of agar and 5 grams of peptone (the peptone may be dissolved in Solution A, if preferred) in 500 cc. of distilled water. Make up the water lost by boiling and cool to 50° C.

Mix the two solutions. Adjust the pH to 6.8 to 7.0, using either a colorimetric (bromthymol blue) or an electrometric procedure. (Material for pH determinations can be secured by heating a small amount of the medium to coagulate the protein and then filtering or pipetting off the clear liquid.) Distribute the agar in flasks (one liter in a two-liter flask is satisfactory), autoclave at 15 pounds for 45 minutes and filter, tube, sterilize or stock without filtering.

*Dilutions and Plating.* Where the character of the butter is not known dilutions of 1:100, 1:1,000, 1:10,000, and 1:100,000 are suggested. Where the character of the butter is known, the number of dilutions may be reduced, but in no case should less than two dilutions be made for each sample.

Dilution bottles similar to the types that are recommended for milk analysis should be used. The dilution water should be at 40 to 45° C. and in dilutions of 1:100 the medium should be poured before the butter congeals, so as to obtain an even distribution of the sample throughout the medium within the plate.

*Incubation.* Four to five days at 21 to 25° C. plus 2 days at 37° C.

*Counting.* The same procedure as in counting milk plates should be followed, using those plates that contain from 30 to 300 colonies. All colonies on such plates should be counted. Exceptions should be made only in those cases where the lowest dilution plated contains less than 30 colonies and where the highest dilution contains more than 300 colonies.

*Expression of Results.* The results as to the number of colonies per cubic centimeter of butter should be expressed as the total count. The medium used and the temperature of incubation should be reported.

### PROTEIN DIGESTING BACTERIA

There are two methods suggested for the determination of protein digesting bacteria in butter. They are:

1. By picking colonies from the plates that have been used to determine the total number of bacteria in butter and inoculating these colonies into tubes of sterile skimmilk containing either brom-cresol-purple or litmus.

*Preparation of Brom-cresol-purple Indicator Solution.* One gram of dibromo orthocresolsulfonphthalein should be ground fine in a glass mortar and 28 cc. of N/10 sodium hydroxide added and the mixture stirred well, the mixture then diluted to about 180 cc. with distilled water, shaken until the solution is complete and then made up to 200 cc. with distilled water. A satisfactory concentration for the coloring of milk is about 0.005 per cent and is obtained by adding 10 cc. of the above solution per liter of milk, imparting to the milk a deep glaucous gray color (5).

All the colonies should be picked from a plate or contiguous colonies from a given sector of a plate.

Incubation of tubes should be at least for 5 days at 21° C.

*Expression of Results.* Results are to be expressed as the proportion of the colonies picked that show proteolysis in milk.

2. By using a medium that contains casein and noting the proteolysis as it occurs on the plate.

The following media are suggested:

A. Beef Infusion Agar plus milk:

Add to each plate 0.5 cc. of sterile skimmilk at the time of pouring. More milk may be added if desired but with 0.5 cc. per plate the proteolytic types stand out very clearly.

B. Frazier and Rupp Casein Agar: (9)

*Preparation of Frazier and Rupp Casein Agar.* Soak 3.5 grams of casein (according to Hammersten) for 15 minutes in 150 cc. of distilled water, then add 72 cc. of saturated lime water. Shake the mixture until almost dissolved, add 0.35 grams of potassium citrate, and continue shaking till casein is dissolved. Then add 10 cc. of double strength beef infusion and make up to 300 cc. with distilled water. To this solution are added 100 cc. of a 0.15

per cent calcium chloride solution and 100 cc. of a phosphate solution which contains 0.105 per cent Sorensen's phosphate ($Na_2HPO_4 \cdot 2H_2O$) and 0.035 per cent $KH_2PO_4$ and which has a pH value of 7.4. The resulting 500 cc. of casein solution is divided into 50 cc. portions in 200 cc. flasks and autoclaved at 20 pounds pressure for 15 minutes. The resulting pH value should be 7.0. Likewise, 50 cc. portions of 3 per cent washed agar are sterilized in separate flasks. When the medium is to be used the casein and the agar solution are heated and while hot the agar poured into the flask of casein solution and thoroughly mixed. Plates are then poured with the milky solution which results.

After incubation, flood the plates with dilute acid (1 part concentrated hydrochloric acid to 10 parts of water). If clearing remains, it denotes true hydrolysis of the casein, if not, it is a weak acid clearing.

*Incubation.* Four to five days at 21 to 25° C.

*Expression of Results.* The number of proteolytic colonies per cubic centimeter of butter should be reported with a record of the medium and procedure used.

### DETECTION OF LIPOLYTIC ORGANISMS

A procedure for the detection of fat hydrolysis by bacteria has been described by Turner (12). Hussong (13) has studied this procedure and recommends as follows:

The emulsion of fat is most easily made by using 0.5 per cent plain agar to which has been added 2–4 per cent of butterfat or olive oil (100 cc. quantities). The agar is allowed to solidify after sterilization and the mixture then warmed sufficiently to melt the particular fat being used. Emulsify by shaking vigorously.

Nile-blue sulfate is used in one of the following ways:

(1) Add 0.5–1.0 cc. of a 0.1 per cent sterile aqueous solution directly to each petri dish before the nutrient agar is added.

(2) Add the dye to the nutrient agar before sterilization.

The above medium may be used for direct plating or for smearing on the surface to determine if the organisms are lipolytic.

If pouring a number of plates it is more convenient to add the nile-blue sulfate to 100 cc. lots of infusion agar than to test tubes. The sterile fat emulsion is added to the agar just prior to pouring.

Lipolytic bacteria turn the fat globules beneath and around the colony blue. Nile-blue pink (an impurity which is present in nile-blue sulfate) causes the unhydrolyzed fat globules to appear pink. Some small colonies and parts of large colonies may absorb the dye and become blue and this phenomenon should be distinguished from a change in the fat globules themselves.

*Incubation* is 3 days at 21–25° C.

*Expression of Results.* Number of colonies per cc. of butter which cause a blue coloration of the fat globules.

### A METHOD FOR THE MICROSCOPIC EXAMINATION OF BUTTER

Hammer and Nelson (6) have developed a method for the microscopic examination of butter and the following is taken from their report.

1. The method of sampling butter is the same as for plate analysis. The entire sample of butter, or enough of it to yield 10 cc. of the melted product for centrifuging, is put into a beaker so that it can be readily melted and mixed. The melting and mixing are carried out by placing the beaker in a water bath at a temperature of from 50 to 55° C. and slowly agitating the butter, using a thermometer as a stirring rod, until the temperature reaches 45° C.

2. Ten cc. of the melted butter at a temperature of 45° C. are transferred to a 30 cc. separatory funnel and centrifuged, using a pipette that has been warmed by passing it through a flame. The size of the portion centrifuged is of no great importance but a standard volume seems advisable and one of 10 cc. has some advantages over smaller amounts. These advantages are, (1) enough serum collects to permit the removal of the serum without including any considerable percentage of fat, and (2) the volume of serum secured provides ample material for plating in case the microscopic picture makes this advisable.

The centrifuging should throw out the serum completely without causing a separation in the serum itself. When centrifuging has been done properly the fat layer will be clear and the serum layer uniform throughout. With a centrifuge in which the stop-cocks of the funnels were 14½ inches apart with the machine in operation, centrifuging 1 minute at a speed of 1,000 r.p.m. was found to be entirely satisfactory for the types of butter used. Samples of butter of different characters behave differently, and some types are easily over-centrifuged so that there is a separation in the serum. When this occurs a solid pack of material at the bottom of the funnel may make it very difficult to start a flow through the stop-cock and, of course, it becomes imperative to remove the serum very completely and then thoroughly mix it, if a representative portion is to be secured for spreading on the slides. In general, centrifuging of butter made from a poor quality of cream must be carefully done or there will be a separation in the serum; presumably, this is due to the content of particles of casein or some casein derivative that readily centrifuges out. With butter made from good quality sweet cream the centrifuging can be greatly in excess (either time or speed) of that suggested without any undesirable effect.

3. Immediately after centrifuging, the funnel is removed from the centrifuge and the serum drawn off (Fig. 1);[2] unless this is done before

[2] Secured from A. H. Thomas Company, Philadelphia, Pa.

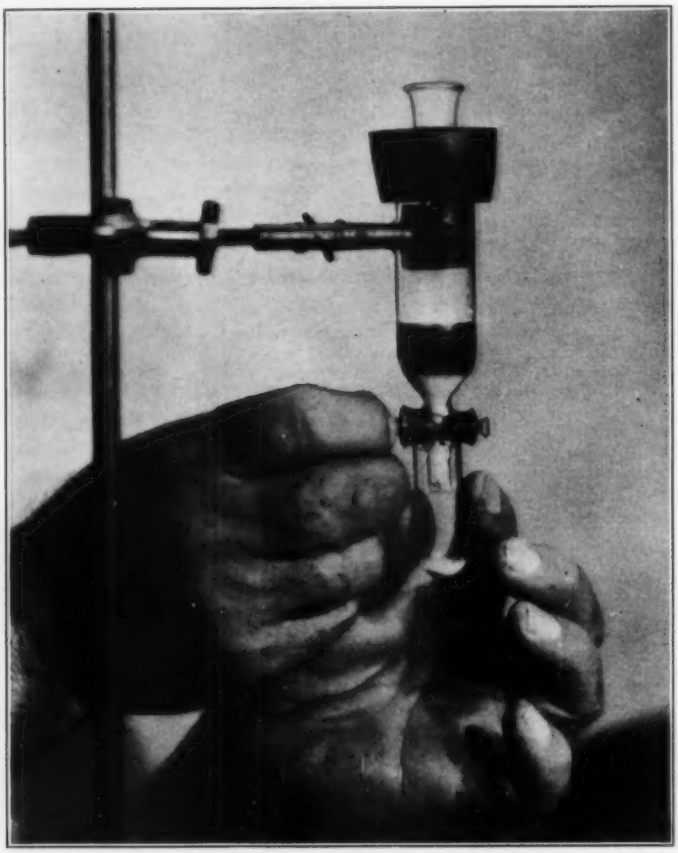

FIG. 1. REMOVING THE SERUM FROM THE SEPARATORY FUNNEL.

the fat begins to congeal, a great deal of difficulty is encountered. The serum should be removed very slowly so that the plane between the fat and serum is kept as flat as possible. When the serum is drawn off rapidly the portion along the wall of the funnel tends to lag and the fat is pulled down to the stop-cock when there is still a considerable percentage of the serum in the funnel. The serum is removed as completely as possible without including objectionable amounts of fat.

A short test tube makes a very satisfactory container in which to collect the serum since portions of the serum can be easily removed from it with a Breed pipette.

4. The collected serum is thoroughly agitated, and portions are removed to microscope slides with a Breed pipette. Ordinarily 0.01 cc. of the serum

is spread over 1, 4, 8 or 9 sq. cm. If the area is larger than 1 sq. cm., sterile skimmilk, free from stainable bacterial cells, or water is added with a loop to facilitate even distribution. The spreading is conveniently done with a transfer needle bent at an angle of 90 degrees. When skimmilk or water is to be used it should be put near the 0.01 cc. of serum and thoroughly mixed with the serum before an attempt is made to cover the desired area.

The area over which the 0.01 cc. of serum should be spread depends on the bacterial content of the butter and the information desired. If a study of the morphologic types of organisms present is the main consideration it is an advantage to have the organisms rather numerous in each field while if an estimation of the number is desired the organisms should not be so numerous that counting is difficult. With butter having a very high bacterial content, counting may be facilitated by spreading the 0.01 cc. of serum over an area larger than 9 sq. cm.

A card with the desired area laid out in black and surrounded by a broad red border is a great aid in the making of spreads that are reasonably exact in area and reasonably uniform in thickness. If a portion of the background is white the desired results are not so easily secured.

The slides are rapidly dried on a level surface and are well protected from flies and dust.

5. Both the regular methylene blue stain described in Standard Methods of Milk Analysis (1) and Newman's one-solution technique are satisfactory for staining preparations of the serum secured from butter.

A. The technic as given in Standard Methods of Milk Analysis (1) is as follows:

"After drying, the slides are to be dipped in xylol, or any other suitable fat solvent, for a sufficient time to remove the fat (at least one minute), then drained and again dried. After this, the slides are to be immersed in 90 per cent grain or denatured alcohol for one or more minutes, and then transferred to methylene blue stain. Old or unfiltered stains are to be avoided as they may contain bacteria and troublesome precipitates."

The slides may be dipped in the stain just long enough to stain them properly or they may be overstained. They are then rinsed in water and may be decolorized in alcohol if they are overstained. The methylene blue dye used for preparing the stains shall be that certified by the Commission on Standardization of Biological Stains. It is recommended that the staining solution be prepared by adding 0.3 grams of methylene blue (90 per cent dye content) to 30 cc. of 95 per cent ethyl alcohol (denatured alcohol is ordinarily suitable for this purpose) and add to this solution 100 cc. of distilled water. When properly decolorized the background of the film should show a faint blue tint. Poorly stained slides may be decolorized

and restained without injury. After drying the slides may be examined at once, or they may be preserved indefinitely.

B. The Newman one solution technic (10).

| | |
|---|---|
| Methylene blue powder, certified | 1–1.12 gm. |
| Ethyl alcohol, 95 per cent | 54 cc. |
| Tetrachlorethane, technical[3] | 40 cc. |
| Acetic acid, glacial | 6 cc. |

Add alcohol to the tetrachlorethane in a flask and heat to a temperature not to exceed 70° C. (if it is desired to use methyl alcohol, the temperature should not be raised to more than 55–60° C.). Add the combined solution to the powdered methylene blue. Shake vigorously until the dye is completely dissolved. The original directions state that the glacial acetic acid should be added at this point, but Mr. Newman writes that it is better to cool the solution before adding acid and that the acid should be added very slowly. Further heating of the acid solution should be avoided. Filter and keep in a tightly stoppered bottle.

*Directions for Use*

1. When dry, immerse the smear in the solution and withdraw immediately.
2. Drain until dry (30 seconds).
3. Wash in water.
4. Dry and observe.

This solution yields a clear, well stained preparation. The smears readily wash off the slide unless they are dried before washing (Step No. 2). If dried too rapidly the smears present a spongy appearance. If the smears are dipped in 70 per cent alcohol, this trouble may be prevented.

6. The examination of the stained smears under the oil immersion lens shows the general morphologic types and the general number of organisms present.

7. The number of organisms per cc. of butter may be estimated by (1) determining the average number per field, counting as many fields as seems desirable for the purpose in mind; (2) calculating the number per cc. of serum from the area over which 0.01 cc. of serum was spread and the diameter of the microscopic field; and (3) dividing by 9[4] to change from a basis of 1 cc. of serum to a basis of 1 cc. of butter. Steps 2 and 3 may be com-

[3] Obtainable from Eastman Kodak Company, Rochester, N. Y.

[4] With the lots of butter having moisture contents that would be acceptable in a well-managed butter plant, the volumes of serum secured from 10 cc. of butter regularly equalled 1.1 cc., or approximately one-ninth of the volume of the butter; accordingly, the bacterial content per cc. of serum should be divided by nine ($10 \div 1.1 = 9$) to get the results on a basis of 1 cc. of butter.

bined for routine work. The same results can be secured by spreading 0.01 cc. of serum over 9 sq. cm. area.

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## THE CHEMICAL ANALYSIS OF BUTTER

The recognized value of the directions for operating the Babcock test and of the specifications for Babcock test glassware promulgated by the committee on this method for the American Dairy Science Association prompted an extension of this type of activity. The methods are considered primarily from the view-point of accuracy and standardization of testing procedures within the industry. The work will be of greatest value if those concerned with the use of these tests will express their views to the chairmen of the various committees so that methods can be adjusted to give most general satisfaction. The following report constitutes the second procedure recommended through the committees on chemical methods. The method is adapted to the complete analysis of butter in the laboratory rather than for a rapid churn test.

The two general methods of making a complete analysis of butter in a creamery are: the procedure of the Association of Official Agricultural Chemists (A. O. A. C.), which is used by the Federal Government and State Chemists; and the modified Kohman test, which is simpler of operation and which better satisfies the creamerymen.

At present, only a few butter manufacturing plants are regularly making either daily or weekly analyses of butter for milkfat, water, salt, and casein. Most creameries make churn tests for water and salt and the milkfat is obtained by difference, after making an allowance for casein. The time is at hand when all creameries ought to know the complete analysis of their butter and a sufficient number of complete analyses should be made to make certain that the milkfat content is correct as secured by difference. This practice is followed at the present time in the better class of plants.

### THE MODIFIED KOHMAN METHOD OF ANALYZING BUTTER

So long as the modified Kohman method of analyzing butter is more simple of operation than the A. O. A. C. procedure, it is being given preference for the operator of a creamery. And in order to particularly assist the creameryman, who is a layman in matters pertaining to chemical analysis, a complete list of equipment and supplies is hereby given, and a discussion of the operation of the test follows.

#### *Equipment and supplies*

A large spatula and a small spatula. The larger one may be made of hardwood, about $\frac{1}{16}$ by $\frac{1}{2}$ by 10 inches in dimensions. The smaller one should be of thin metal. A pocket knife will suffice.

A sample jar, in which the butter is thoroughly stirred to a creamy

FIG. 1. EQUIPMENT AND SUPPLIES FOR THE MODIFIED KOHMAN METHOD OF ANALYZING BUTTER

consistency, which may be a pint milk bottle fitted with a number 9 rubber stopper. For an alternative method of preparing a butter sample, a pint fruit jar that is fitted with a rubber ring may be employed. In this second procedure (1) the butter is melted and then it is vigorously shaken during the cooling process.

A metal beaker or dish approximately 2 inches in diameter and 3 inches high. A small pair of forceps to handle the weights and to shove the riders back and forth on the beams. A larger pair of forceps, for lifting the beaker, was unintentionally left out of figure 1.

A set of weights weighing from 1 mgm. to 100 grams, that costs about $5. A torsion balance valued at about $40, which may be kept in a home-made cabinet. A tripod with an asbestos mat. An alcohol lamp. A box of matches. A supply of gasoline, such as is found in all automobile filling stations. A thin metal shelf. A cooling surface such as an old flatiron or a smooth building stone, or concrete.

A roll of tissue. A rubber-tipped glass rod or soft-wood stirrer. A supply of silver nitrate. A burette. A bottle of potassium chromate. A pipette. A graduated cylinder or a flask. A white cup and a stirring rod with a color scale is convenient.

*Operation*

1. Obtain the sample. When sampling from the churn the hardwood spatula is satisfactory. The extraneous moisture may be eliminated by scraping off the surface of the butter in the churn, over a small area, and immediately obtaining a portion of approximately 10 to 15 grams. For every-day churn analysis, a sample should be made up of 10 or 12 portions. This butter should be placed in a jar that has been dried over a steam pipe or in a similar way, for at least several hours. The type of jar will depend on the method employed in preparing the sample. When the butter is thoroughly mixed by stirring, a pint glass milk bottle properly fitted with a rubber stopper is satisfactory, for the neck is long and sloping. In a milk bottle, small portions of butter can not stick in inaccessible places, as is true of bottles with square shoulders.

When the sample is taken from the package and not from the churn, a portion should be obtained from each tub or printer box. This should give a representative sample of the finished product of a single churning. If the sample is obtained during the packing process a portion of soft butter may be obtained from each package before it is finished off. When the sample is obtained from hard butter, a single trierful taken by boring diagonally from the top to the bottom will suffice. About an inch of butter from each end of the trier, in this case, should be eliminated.

2. Balance the metal beaker or dish, placing the metal disc or a watch glass over the top. The doors of the cabinet, during weighing, should be at

right angles with the case, so that the balances may not be affected by drafts of air.

3. The sample of butter should now be prepared for weighing. When the butter is brought to a creamy consistency by stirring, it should be warmed in water at a temperature from 100° to 110° F. until the milkfat has almost reached the melted stage. In case the test does not have to be completed quickly, the butter may be softened at room temperatures. If care has been exercised in warming the sample, so that it is not too soft, it will not be necessary to cool it during the latter part of the preparation. Thus, time may be saved. When a sample of butter is being prepared, it should be tightly closed, with the exception of when it is being stirred. During the weighing the jar should be made tight as quickly as the butter has been taken out.

A mechanical stirrer is being employed in some large laboratories. One of these agitators is a small U-shaped tip on the end of a shank that fits a small electric drill. This U tip is about ¾ inch wide and 1 inch long. It is made of strap iron that is approximately ½ inch wide.

4. By the employment of the large hardwood spatula, place approximately 10 grams of butter in the beaker or dish. The small metal spatula or pocket knife should be used here, to scrape the butter from the large one into the dish. The butter should be landed on the bottom of the dish, and not smeared over the sides. The toilet tissue is convenient to wipe the butter from the spatula, the knife, and other apparatus.

Time should not be taken to weigh exactly 10 grams of butter into the dish, for there will be too much evaporation of moisture before the weight is obtained. The metal disc or watch glass should be placed on the dish as quickly as the butter is delivered into it. This precaution in weighing makes necessary the use of the block of weights that weigh to 0.01 gram. In case the butter-maker wishes only a close approximation of the composition of his butter, he can save time by weighing out exactly 10 grams of butter. Then the percentage moisture can be read directly on the beams of the scale. Thus, he is saved the time of computing the percentage. To illustrate the obtaining of the amount of butter in the former case, let us say that the beaker weighed 25.48 grams, and that the beaker and the butter amounted to 35.12 grams. The difference is the weight of the butter, $35.12 - 25.48 = 9.64$ grams of butter.

5. By the use of the large forceps transfer the dish from the balances to the asbestos pad, in case a flame of gas or alcohol is used to drive off the moisture, or to the hot-plate if electricity is the heating medium. During the drying process, it is desirable to use an asbestos pad over a flame, for otherwise carbon is likely to deposit on the dish and affect the weight. The employment of rather low temperatures, such as 275° to 300° F. is essential, for at higher temperatures the butter is likely to spatter out of

the dish. At this time it is wise to shake the samples sufficiently often to completely break up the casein blanket. When this casein surface is not properly broken, particles of it will float away later, on the gasoline. This causes an error in the analysis.

6. As quickly as the milkfat has reached a golden-brown color, the dish should be placed, with the forceps, on a smooth and clean iron or stone surface to cool. The forceps should be employed in preference to the hands, for the grease or moisture of the hands might change the weight of the dish. Iron or stone, which have an extremely high capacity of heat conduction, will rapidly cool the beaker and its contents. This cooling will be accomplished in 5 minutes, which is about the same as the time consumed in reducing the temperature over an electric air-fan or by cooling in cold water. When the last method is followed, there is danger of not removing all the moisture from the dish, even though it is wiped with a dry cloth. It is usually advisable to place a weight on the dish or beaker when it is cooling, so that its contact with the iron will be intimate. The cooling iron should be free from dust, grease, and the like, in order that the weight of the dish will not be affected.

7. The cooled beaker and its contents weighed 33.63 grams. 35.13 grams (weight of beaker and butter in step 4) − 33.63 grams = 1.49 grams (weight of evaporated moisture). The percentage of moisture is $\frac{1.49 \text{ grams} \times 100}{9.64} = 15.45$.

8. Fill the cup to within about ¾ inch from the top with gasoline, and thoroughly stir it with either a rubber-tipped glass rod or a softwood spatula. The purpose of the gasoline is to dissolve the milkfat. After it has stood approximately three minutes, the salt and casein will have settled to the bottom. Then the gasoline with its load of milkfat may be carefully poured into the drain. The beaker must be handled gently, so that the salt and casein will remain at the bottom. If little flakes of casein float on the surface of the gasoline, they are an indication that the casein film was not properly broken by agitation during the process of the evaporation of the moisture, as pointed out in step 5.

9. Again fill the beaker with gasoline and thoroughly stir the mixture. After standing another three minutes this gasoline may be poured off. Where it is wise to be economical in the use of gasoline, this second washing may be saved, to be used in the first extraction in the succeeding analysis. At this stage about 2 cc. of gasoline will remain in the beaker.

10. If an electric hot plate is employed, the beaker or dish with the remaining salt and curd may be dried by placing it immediately on the plate. In case the evaporation must be accomplished over a flame of gas or alcohol, the remaining gasoline should be largely dried off over a steam pipe or a radiator, for gasoline is too inflammable to be placed directly over a flame

at this stage of the analysis. The thin metal shelf shown in figure 1, may be placed over the steam line for convenience in holding the beaker at this period of the process. The completion of the drying process may be accomplished over a low flame when an asbestos pad is employed. The contents of the beaker when evaporation is completed, should be largely in the form of a dry powder.

11. Cool the beaker and its contents and then weigh. The calculation of the amount of milkfat is done as follows: 33.63 grams (weight before milkfat was extracted) – 26.19 grams (weight after milkfat was extracted) = 7.84 grams of milkfat.

The percentage of milkfat is $\frac{7.84 \text{ grams} \times 100}{9.64 \text{ grams (weight of butter)}} = 81.32.$

FIG. 2. THE TROY SALT TEST

12. In preparation for determining the amount of salt, transfer all of the contents of the beaker to the 300 cc. flask, in case of the Troy salt test, or a 250 cc. flask if the Nafis salt test is employed. This transfer may be accomplished by filling the beaker approximately three-fourths full of lukewarm water and stirring the mixture with a rubber-tipped glass rod or a softwood spatula. Then after pouring the contents of the beaker into the flask, the procedure should be repeated until all of the salt and curd have been transferred to the flask. Now the flask should be filled to the 300 cc. or 250 cc. mark with warm water.

13. Shake the flask sufficiently long for the salt to be uniformly distributed throughout the solution. Now measure out 17.6 cc. of the salt solution when the Troy salt test is being used, or 25 cc. if the Nafis test is being employed; and put it into a white cup for titration.

14. After putting 2 or 3 drops of indicator (potassium chromate) into the salt solution in the white cup, titrate into it the silver nitrate from the

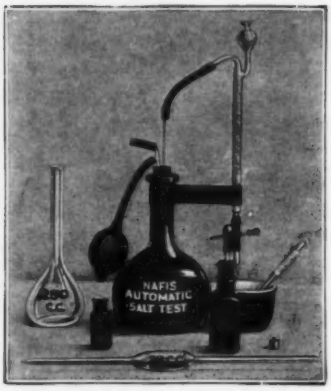


FIG. 3. THE NAFIS SALT TEST

burette to a brownish-red color. In the Troy salt test the silver nitrate is a tenth normal solution, and in the Nafis salt test it is a special strength but in both cases the dilutions are calculated so that the cubic centimeters of silver nitrate used is equal to the percentage of salt. In this analysis it was 2.5 per cent.

15. The following procedure is followed in calculating the percentage of curd. The sum of the moisture, 15.45 per cent, milkfat 81.32 per cent, and salt 2.5 per cent, is 99.37 per cent. 100 per cent – 99.37 per cent = 0.63 per cent curd.

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# American Dairy Science Association Announcements

## ANNUAL MEETING

JUNE 26–29, 1933, AT THE UNIVERSITY OF ILLINOIS, URBANA

The program committee in its statement of March 21 emphasized that Urbana is only 126 miles from Chicago by rail and 138 miles by good auto road. The meeting of the Association and Century of Progress Exposition in Chicago can be combined as a vacation trip.

Members are urged to bring their families and events are being arranged for the ladies and children. Rooms with running water in dormitories will average $2.25 per person for the three days while the rates at hotels in Champaign and Urbana are $1.50 and up per person per day. Headquarters during the meeting will be Newman Hall, corner of Armory and Sixth Streets, in Champaign.

If reservations have not been made communicate at once with H. A. Ruehe, Department of Dairy Husbandry, Urbana, Illinois, giving number in party and number of women and children, dormitory or hotel preference, and date of arrival and departure.

Papers, except by special invitation, will be limited to members but, in accordance with past custom, the meeting will be open to all who are interested in attending.

### *Meeting of Southern Division*

The Southern Division held its annual meeting on Feb. 1 and 2 in New Orleans. Chairman R. B. Becker, of Florida, presided. Papers were presented by A. J. Glover, of Wisconsin, on dairying in agriculture; by J. R. La Master and E. C. Elting, of South Carolina, by A. H. Kuhlman and E. Weaver, of Oklahoma, and by J. L. Fletcher and R. H. Lush, of Louisiana, on various aspects of pasture problems; by J. S. Moore, of Mississippi, on dairy rations; by O. C. Copeland, of Texas, and F. R. Edwards, of Georgia, on sorghum and corn silage; by R. B. Becker, of Florida, on preparation for service; by C. H. Staples, of Louisiana, on use of college herds; by W. H. Eaton, of Alabama, on rations; by E. W. Neasham and A. J. Gelpi on Louisiana dairy manufacturing problems; by J. I. Keith, E. Fouts, and C. W. Rink, of Oklahoma, on new methods of manufacturing cheddar cheese; by A. D. Burke, of Alabama, on chocolate ice cream; and by F. W. Bennett, of Georgia, on contamination of equipment during manufacture of butter.

The ballot by mail resulted in the election of A. D. Burke, of Alabama, as chairman, R. H. Lush, of Louisiana, as vice-chairman, and E. C. Elting, of South Carolina, as secretary.

In commenting upon dairy conditions in the south, R. B. Becker stated in part:

"Trends in the southern dairy industry show that from 1926 to 1932, production of butter increased 39.2 per cent; cheese increased 3726.9 per cent; condensed and evaporated milks 434.1 per cent; and powdered milk products 1268.3 per cent. On the other hand, factory production of ice cream decreased 22.1 per cent; condensed buttermilk 37.9 per cent; and renovated butter 11.1 per cent, thanks to work on the care of milk and cream.—On January 1, 1927, we numbered 74 workers in the field of dairying—increased to 92 at the beginning of the current year.—The physical facilities include new dairy manufacturing class rooms and laboratories at Alabama, Kentucky, Louisiana, Oklahoma and Virginia; with major improvements of existing facilities at Arkansas, Georgia, Mississippi, South Carolina, Texas and West Virginia. New barns, silos, or additional housing space for dairy cattle have been provided at Alabama, Arkansas, Florida, Louisiana, Mississippi, the Carolinas, Oklahoma, Texas and West Virginia."

### *Dairy Congress in Italy*

Word has been received from Dr. H. H. Boysen of the Dairy Institute near Berlin that the next International Dairy Congress that was to have been held in Berlin in 1934 is to be held at Rome, Italy, in 1934. German dairy workers hope that conditions in Germany will be sufficiently adjusted to permit holding the Dairy Congress in Berlin in 1937.

The Congress of the International Society of Microbiology that was to be held at the Robert Koch Institute in Berlin this summer has been postponed and plans are being made to hold this Congress in Berlin during June or July of 1934.

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## CLARENCE HENRY ECKLES

The death of Doctor Clarence Henry Eckles was a notable loss to the dairy industry. He served the industry by investigations in both dairy cattle and dairy manufacturing problems of a practical and of a strictly scientific aspect. He was preeminent in the development of men to serve in all branches of the dairy industry with ability which was a credit to their teacher. He recognized the need for and was a pioneer in the development of science which furnished facts to assist in rounding out our dairy and scientific knowledge that the solution of new problems might be visualized.

Clarence Henry Eckles was born on a farm in Iowa on April 14, 1875. He died in St. Paul, Minnesota, on February 13, 1933. He was graduated

from Iowa State College in 1895 and later studied at the University of Wisconsin, and at the Massachusetts Agricultural College. From 1901 to 1919 he was head of the dairy department at the University of Missouri and from 1919 he was head of the dairy department at the University of Minnesota. In 1904 and 1905 he studied in Germany and Switzerland, his specialty being bacteriology.

The American Dairy Science Association and its publication the JOURNAL OF DAIRY SCIENCE are deeply indebted to him for its development. He was charter member of the former official Dairy Instructors' Association which developed in its scope and objects and became the American Dairy Science Association. He served as its president and in innumerable less conspicuous capacities. He was active in promoting the JOURNAL OF DAIRY SCIENCE and as a member of the board of associate editors his counsel helped to formulate present JOURNAL policies.

A more comprehensive tribute to him will be found elsewhere in this number.

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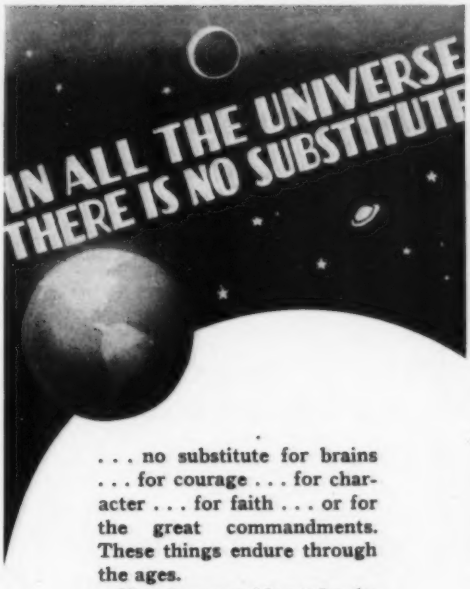


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